LAND USE AND LAND COVER CHANGE: THE EFFECTS OF WOODY PLANT ENCROACHMENT AND PRESCRIBED FIRE ON BIODIVERSITY AND ECOSYSTEM CARBON DYNAMICS IN A SOUTHERN GREAT PLAINS MIXED GRASS SAVANNA

A Dissertation

by

EMILY BROOKE HOLLISTER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2008

Major Subject: Molecular and Environmental Plant Sciences

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ABSTRACT

Land Use and Land Cover Change: The Effects of Woody Plant Encroachment and Prescribed Fire on Biodiversity and Ecosystem Carbon Dynamics in a Southern Great Plains Mixed Grass Savanna. (May 2008)

Emily Brooke Hollister, B.A., Wells College

Chair of Advisory Committee: Dr. Thomas W. Boutton

In the southern Great Plains, the encroachment of grassland ecosystems by mesquite (*Prosopis glandulosa)*, is widespread, and prescribed fire is commonly used in its control. Despite this, substantial quantitative information concerning their influences on the community composition, functional dynamics, and soil organic carbon (SOC) storage potential of grassland ecosystems is lacking. The objectives of this study were to: a) quantify the effects of seasonal prescribed fire treatments and mesquite encroachment on aboveground net primary productivity (ANPP) and herbaceous community composition; b) characterize SOC pool sizes, turnover, and storage potential relative to vegetation type and fire treatment; c) evaluate the structure and diversity of soil microbial communities relative to vegetation type; and d) characterize the functional diversity of these same microbes using the GeoChip functional gene microarray.

Repeated winter and summer fires led to increased ANPP rates (average, 434 and 313 g m^{-2} y^{-1}, respectively), relative to unburned controls (average, 238 g m^{-2} y^{-1}), altered herbaceous community composition, and increased the storage of resistant forms of SOC, but did not affect overall SOC storage. Herbaceous ANPP rates did not differ significantly as a result of mesquite encroachment, but herbaceous community composition and SOC storage did. Mesquite soils contained significantly more total, slow-turnover, and resistant forms of SOC than those that occurred beneath C_3 or C_4 grasses. Similarity among the soil bacterial and fungal communities associated with the major vegetation types in this system was low to moderate. Significant differences were detected among soil fungi, with the mesquite-associated fungi harboring significant

differences in community structure relative to the fungal communities associated with each of the other vegetation types examined. Despite this result, few significant differences were detected with respect to the functional diversity of these communities, suggesting either a high degree of functional redundancy, or that the functional differences harbored by these communities are beyond the scope of the GeoChip. The results of this study demonstrate that both fire and mesquite encroachment have the potential to alter ecosystem components and processes significantly, providing new insight regarding the effects of these widespread land use and land cover changes on ecosystem structure and function.

ACKNOWLEDGEMENTS

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CHAPTER I

INTRODUCTION

Land use and land cover changes frequently alter key aspects of ecosystem structure and function, affecting above- and belowground dynamics and contributing to processes of global change (Schlesinger 1997, Houghton and Goodale 2004, Jarnagin 2004, Chapin et al. 2005, Foley et al. 2005). Among the most common land use and land cover changes affecting grassland systems today is that of woody plant encroachment. Having been documented in North and South America, as well as Australia, Africa, and Southeast Asia (Van Auken 2000, Archer et al. 2001, Jackson et al. 2002, Liao et al. 2006b), the woody encroachment of grassland systems is a geographically extensive phenomenon that has the potential to alter global nutrient cycles dramatically. Grassland systems cover approximately 40% of terrestrial land area and account for 30% of soil organic carbon on a global basis (Scurlock and Hall 1998), thus changes to their structure and function are likely to have impacts at the global scale.

Over the course of the last century, dramatic increases in the density and distribution of *Prosopis glandulosa* Torr. (honey mesquite) have occurred in the mixed grass prairies of the southern Great Plains (USA) (Ansley et al. 2002, Asner et al. 2003). The encroachment of this nitrogen-fixing, woody species often results in increased levels of above- and belowground plant biomass, as well as enhanced stores of carbon and nitrogen in plant tissues and soils (Hibbard et al. 2001, Liao et al. 2006b). Although the potential for carbon storage may been seen as a beneficial consequence, mesquite encroachment has also been linked to reduced abundances and productivity of native grassland species (Archer et al. 2001, Ansley and Castellano 2006). Thus, mesquite encroachment has come to represent a major challenge to landowners and natural resource managers, often requiring substantial investments of money, time, and energy

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This dissertation follows the style of Ecology.

in order to maintain the condition of their properties, as well as the economic viability of their livestock production, hunting, and/or ecotourism enterprises (Scifres and Hamilton 1993).

Seeking to control or slow the spread of mesquite and other woody species, landowners often employ mechanical removal methods and/ or herbicide treatment in the management of mesquite-encroached rangelands, but, in recent years, the use of prescribed fire has begun to emerge as a popular, lower-cost alternative (Archer et al. 2001, Teague et al. 2001). Fire tends to reduce the biomass of woody plants and other invasive species while having a stimulatory effect on the productivity of native grasses (Wright and Bailey 1982, Collins and Wallace 1990), and when timed appropriately, it has the potential to stimulate the growth and productivity certain species in favor of others (Howe 1994, 1995, Engle and Bidwell 2001).

Both fire and woody encroachment have the potential to influence ecosystem patterns and processes both aboveground and belowground, as well as at multiple scales. Despite the common occurrence of woody encroachment as a land cover change phenomenon and the increasingly common use of fire as a land management tool, substantial quantitative information concerning their ability to influence community composition and function, as well as the carbon storage potential of grassland ecosystems, particularly those of the southern Great Plains, is lacking, or is at best, conflicting. For example, the woody encroachment of grassland systems has been shown to result in the increased accumulation of organic carbon in the soil (San Jose et al. 1998, Tilman et al. 2000, Pacala et al. 2001, Liao et al. 2006b, Zavaleta and Kettley 2006), no net change in soil organic carbon concentrations (McCarron et al. 2003, Smith and Johnson 2003, Hughes et al. 2006), and even carbon loss (Jackson et al. 2002, Hudak et al. 2003). Likewise, the effects of fire on carbon cycling and soil organic carbon storage have also been shown to vary widely, with responses ranging from increased soil organic carbon storage (Ansley et al. 2006a, Dai et al. 2006), to neutral effects (Rice and Owensby 2000, Roscoe et al. 2000), and soil organic carbon loss (San Jose et al. 1998, Bird et al. 2000) following fire treatment.

While somewhat more is known about the effects of fire treatment on plant community composition and productivity, the majority of this knowledge is based upon outcomes of cool season fires in tall grass prairie ecosystems. Such fires have been shown to: a) shift community composition toward dominance by C_4 tallgrass species, b) lengthen the window of the growing season, c) stimulate herbaceous productivity, and d) aid in the control of woody encroachment (Engle et al. 1993, Engle and Bidwell 2001). Much less is known about the effects of warm season fire (Engle and Bidwell 2001, Ansley and Castellano 2007), despite the fact that summertime wildfires are thought to have played an important role in the historic fire regime (Wright and Bailey 1982, Ansley and Jacoby 1998). Likewise, little is known about the effects of fire in southern mixed grass prairie ecosystems. Studies addressing the effects of fire in mixed grass prairies are relatively few in number, and the results of those which do exist in the published literature are often contradictory to one another (Launchbaugh 1964, Wink and Wright 1973, Engle and Bidwell 2001, Ansley et al. 2006b).

Fire and fire-induced vegetation change have the potential to feedback upon and alter other components of the carbon cycle, particularly the composition and activity of the soil microbial community. Soil microbes are closely linked to the structure and function of aboveground plant communities, and they exert considerable control over the carbon and nitrogen cycles in water-limited ecosystems, such as grasslands (Knops et al. 2002). The ability of individual plant species and/or assemblages to support unique microbial communities has been demonstrated in a number of systems (Bardgett et al. 1999, Smalla et al. 2001, Callaway et al. 2004, Grayston et al. 2004, Hawkes et al. 2005, Wallenstein et al. 2007, Broeckling et al. 2008). Such associations are often attributed to differences in productivity rates, nutrient use efficiencies, and the quantity and quality of different species' organic inputs to the soil system. It is thought that changes in the phylogenetic composition of microbial communities may also be accompanied by shifts in their functional diversity and capacity.

The study of soil microbial ecology has expanded rapidly in recent years with the development of cultivation-independent, molecular techniques. Although direct

culturing and gross-level community characterization techniques (e.g. community level physiological profiling and the measurement of soil respiration) have provided important insight regarding the structure and function of soil microbial communities, the scope and utility of these techniques has proven to be limited, as only a small fraction of the soil microbial community is known to be culturable (Torsvik et al. 1990, Broeckling et al. 2008). As such, the use of molecular techniques has become a popular alternative. Analyses of sequence heterogeneity among ribosomal RNA genes have been used to characterize and catalog the composition and diversity of soil bacterial, archaeal, and fungal communities (Pace et al. 1986, Marsh 1999, Schadt et al. 2003, Anderson and Cairney 2004, Schloss and Handelsman 2004, Fierer et al. 2007b), while the detection and quantification of functional genes through techniques such as quantitative- and reverse transcription PCR (Nogales et al. 2002, Burgmann et al. 2003) and functional gene microarrays (Wu et al. 2001, Rhee et al. 2004, He et al. 2007) have provided insight into community functional capacity.

Despite the increased knowledge that has been gained about soil microbial communities in recent years, we still have much to learn regarding: a) the importance and patterns of, as well as controls over, microbial biodiversity and ecosystem processes; b) the relationship that exists between biodiversity and functional trait diversity; and c) the ways in which the dynamics of soil microbial communities may conform to or refute current ecological theory, which is often based upon the relationships and dynamics of macroorganisms (Whitfield 2005, Fierer and Jackson 2006, Fierer et al. 2007a). In addition, the vast majority of molecular-microbial investigations have focused on the bacterial component of the soil microbial community, leaving gaps in our knowledge regarding soil fungal communities, despite the recognition of their critical role in decomposition and nutrient transfer processes in the soil. Increasing our knowledge of both soil fungal and bacterial responses to ecosystem change and global change drivers will be important for understanding and predicting ecosystem function in the future.

This study is an attempt to integrate above- and belowground effects of mesquite encroachment and prescribed fire treatment on community structure and function in a

savanna-like mixed grass prairie ecosystem of the southern Great Plains. This study was approached from a multi-tiered perspective, in which its objectives were to: a) quantify the effects of repeated seasonal fire and mesquite encroachment on rates of aboveground net primary productivity, herbaceous community composition, and plant biomass dynamics (Sala et al. 1981); b) characterize soil organic carbon pool sizes, turnover rates, and storage potential relative to vegetation type and seasonal fire treatment using a combination of long-term incubation, acid hydrolysis, and kinetic modeling techniques (Collins et al. 2000, Paul et al. 2006); c) evaluate the structure and diversity of soil bacterial and fungal communities relative to vegetation type using a direct cloning and sequencing approach (McCaig et al. 1999, Schadt et al. 2003, Anderson and Cairney 2004); and d) characterize the functional diversity of these same soil microbial communities using a functional gene microarray known as the GeoChip (He et al. 2007). The results of this study will provide key information concerning the impacts of woody encroachment and prescribed fire on the dynamics of carbon in grassland ecosystems, the links that tie above- and belowground processes together, and the strength of that connectivity, allowing for an increased understanding of the carbon cycle and the ways in which human activity and global change phenomena impact its components.

CHAPTER II

PRESCRIBED FIRE AND WOODY ENCROACHMENT ALTER ABOVEGROUND NET PRIMARY PRODUCTIVITY IN A SOUTHERN GREAT PLAINS MIXED GRASS SAVANNA

Introduction

Net primary productivity (NPP), the net flux of carbon from the atmosphere into plant tissues, is one of the largest fluxes of the global carbon cycle and underpins a number of critical ecosystem functions (Gill et al. 2002, Scurlock et al. 2002). NPP impacts plant biomass accumulation, the flow of energy to consumers and between trophic levels, and the rates at which key nutrients are cycled (McNaughton et al. 1989, Zak et al. 1994a, Gill et al. 2002). NPP influences the value we place upon ecosystems (Long et al. 1989, Sala and Austin 2000) and, ultimately, shapes land use, management, and conservation practices. Thus, it is important that NPP be quantified accurately, that we seek to improve our understanding of how NPP and other carbon fluxes are controlled (Gill et al. 2002), and that we recognize the role that disturbances, such as land use and land cover change, play in modifying these processes.

Over the course of the last century, dramatic increases in the density and distribution of *Prosopis glandulosa* Torr. (honey mesquite) have occurred in the mixed grass prairies of the southern Great Plains (USA) (Ansley et al. 2002, Asner et al. 2003). The encroachment of this nitrogen-fixing, woody species often results in increased levels of above- and belowground plant biomass, as well as enhanced stores of carbon and nitrogen in plants and soils (Hibbard et al. 2001, Liao et al. 2006b). While light densities of mesquite have the potential to enhance grassland productivity (Heitschmidt et al. 1986, Ansley and Castellano 2006), mesquite encroachment is more often associated with the loss of native grasslands and reductions in biodiversity (Archer et al. 2001). Furthermore, mesquite encroachment has been implicated as playing a role in reducing grassland productivity (Ansley and Castellano 2006) and the economic viability of rangeland livestock production systems (Archer et al. 2001).

Prescribed fire is often used as a means of controlling or slowing the spread of woody plants, like mesquite, and is considered to be an important land management tool. Fire tends to reduce the standing biomass of woody plants and invasive species and is often cited as having a stimulatory effect on native grasses following a period of post-fire recovery (Wright and Bailey 1982, Collins and Wallace 1990). The removal of mulch layers, release of nutrients, stimulation of belowground growth and productivity, and suppression of competition are often identified as mechanisms that may contribute to the increased rates of aboveground herbaceous productivity that are typically observed following fire (Wright and Bailey 1982, Collins and Wallace 1990, Rice and Owensby 2000).

The effects of fire may vary with the season in which it occurs. It is commonly held that community composition may be altered as a result of the season in which fire occurs: "cool season" (i.e. winter or dormant season) fires are thought to shift grassland communities toward dominance by C_4 grass species, while "warm season" (i.e. summer or growing season) fires are thought to promote dominance by C_3 grasses, sedges, and forbs (Engle and Bidwell 2001). This is based upon the reasoning that fire should have damaging effects upon those plants which are actively involved in growth or reproduction when fire occurs (Daubenmire 1968, Howe 1994).

Substantial evidence demonstrating the effects of cool season fires has been observed in the tall grass prairie ecosystems of the Great Plains, where cool season fires have been shown to: (a) shift community composition toward dominance by C_4 tallgrass species, (b) lengthen the window of the growing season, (c) stimulate herbaceous productivity, and (d) aid in the control of woody encroachment (Engle et al. 1993, Engle and Bidwell 2001). Much less is known about the effects of warm season fire (Engle and Bidwell 2001, Ansley and Castellano 2007), despite the fact that summertime wildfires are thought to have played an important role in the historic fire regime (Wright and Bailey 1982, Ansley and Jacoby 1998). Common, though potentially false, assumptions about summer fires suggest that they may compromise the availability of desirable forage species for grazing, have detrimental effects on plant growth and

productivity, or inflict long-lasting changes in community composition (Bragg and Steuter 1996, Engle and Bidwell 2001).

Similar knowledge gaps exist with respect to the effects of fire in southern mixed grass prairie ecosystems. Studies addressing the effects of fire in these ecosystems are relatively few in number. Those which exist in the published literature have focused, in large part, on the effects of cool season burns, and their findings are often contradictory (Launchbaugh 1964, Wink and Wright 1973, Engle and Bidwell 2001, Ansley et al. 2006b). The continued encroachment of woody plants into grassland ecosystems, and the need for effective and economical means for restoration and control, have fueled an increased need to understand the effects of fire and its season of application (Engle et al. 1993, Engle et al. 1998, Ansley et al. 2006b, Ansley and Castellano 2007).

The objectives of this study were to quantify the effects of repeated seasonal fire and woody plant encroachment on rates of herbaceous aboveground net primary productivity (ANPP), herbaceous community composition, and plant biomass dynamics in a savanna-like, mesquite-encroached southern Great Plains mixed grass prairie ecosystem. We hypothesized that: 1) the use of prescribed fire, regardless of season, would lead to increased rates of herbaceous ANPP relative to the unburned control; 2) the season of a fire's occurrence (winter vs. summer) would alter the composition of the herbaceous community such that the contributions of C_4 grasses to ecosystem ANPP would be enhanced following winter fires and contributions from C_3 grasses would increase following summer fires; and 3) the effects of mesquite canopy and prescribed fire would have interactive effects on herbaceous ANPP, resulting in decreased rates of herbaceous community ANPP under mesquite canopies in the unburned controls, but increased rates of herbaceous ANPP in the subcanopy areas of burned plots.

Materials and Methods

Study Site

This study was conducted in a southern Great Plains mixed grass savanna ecosystem, near Vernon, TX (33°51'20" N, 99°26'50" W). Mean annual precipitation at

this site is 665 mm, which is bimodally distributed with peaks in May and September. The mean annual temperature is 16.1^{o} C, and monthly average extremes range from 36^{o} C in mid-summer to -2.5^{o} C in mid-winter (Ansley et al. 1990). The soils at this site are classified as fine, mixed, superactive, thermic Vertic Paleustolls of the Tillman series, with 0 to 1% slope (Soil Survey Staff 2007).

The experimental plots in which this study was conducted were established in 1990 to study ecosystem response to prescribed fire; among these were repeated summer fires (burned during the months of August to September in 1992, 1994, and 2002), repeated winter fires (burned during the months of January to March in 1991, 1993, 1995, and 2002), and a series of unburned controls. Fires were conducted as headfires and were supported by fine fuel loads ranging from 1300 to 4285 kg ha^{-1} (Ansley et al. 2006a, Dai et al. 2006). Each treatment consisted of 3 replicates, ranging from 1 to 6 ha in size. Livestock grazing has been excluded from the site since 1988, and fire records indicate that fire has been excluded from the unburned control plots since the 1960s (Ansley et al. 2006a).

The herbaceous layer vegetation is comprised of a mixture of C_3 and C_4 grass species and forbs, and the overstory is dominated by mesquite. *Nassella leucotricha* (Trin. & Rupr.) Pohl (Texas wintergrass) is the dominant C_3 grass species, and dominant C_4 grasses include the midgrasses *Panicum obtusum* Kunth (vine mesquite) and *Sporobolus compositus* (Poir.) Merr. (meadow dropseed), as well as *Buchlöe dactyloides* Nutt. (buffalograss), a stoloniferous shortgrass species.

Climate Data

Climate data, including long-term averages and daily temperature and precipitation observations over the course of this study, were obtained from the National Climatic Data Center (NOAA 2005) for the recording station at Vernon, TX (Co-op ID # 419346), which is located approximately 30 km from the study site.

Sample Collection and Processing

Herbaceous biomass was harvested from 0.25 m^2 quadrats at approximately 6-week intervals over the course of two years. Live and standing-dead biomass were clipped to ground level and separated into 5 functional types (i.e. C_3 annual grasses, C_3 perennial grasses, C_4 midgrasses, C_4 shortgrasses, and forbs) in the field. Surface litter from each 0.25 m^2 quadrat was also collected. Quadrats were harvested in a paired manner from mesquite subcanopy and open grassland areas. Mesquite sub-canopy was defined as any area within the drip line of the existing canopy. In those plots that were subject to fire treatment, where the canopy may have been destroyed, sub-canopy was defined as the area that fell within the drip line of the remaining dead branches. A minimum of 16 quadrats (8 subcanopy, 8 open grassland) were collected from each experimental fire plot during each of the sequential harvests. Sampling was carried out in a stratified manner to ensure even sampling across all experimental plots and to avoid harvesting biomass from the same location more than once.

Live and standing-dead biomass and litter samples were dried at 60º C for a minimum of 48 hours prior to processing. The retention of green color in the dried plant tissues allowed biomass samples to be separated into live and standing-dead categories, and weights were recorded for each. Litter samples were separated into their herbaceous (i.e. grasses and forbs) and mesquite (i.e. leaves, fruit, branches) components, and weights were recorded. Non-vegetative material collected within litter samples (e.g. insect carcasses, animal feces, or mineral rock or soil) was excluded from this analysis.

Field sample collection was initiated in early-June of 2003, approximately 15 months after the last winter fire and 9 months after the last summer fire were applied. All biomass that was collected in June 2003 was considered to represent the previous month's growth and productivity. As such, year "one" in this study was defined as May/June 2003 through May 2004, and year "two" was defined as May 2004 through May 2005.

ANPP Calculation

ANPP was calculated according to a method described by Sala et al. (1981) and Scurlock et al. (2002), in which positive increments in live biomass that accrue between sequential harvests are considered to represent the net primary productivity that occurred during the inter-harvest period. Such increments are determined on a species- or functional group-specific basis, and the sum of all species or groups is assumed to represent overall net primary productivity. Periods of time in which biomass decreases, or does not change, are considered to represent zero productivity. However, if a decrease in live biomass is accompanied by a concomitant increase in standing-dead biomass, and the sum of these two values is greater than zero, then the remainder (i.e. positive sum) is considered to represent net primary productivity for that time interval.

The ANPP results presented in this study were calculated on the basis of live and standing-dead herbaceous biomass. Plant biomass values were averaged at the plot (i.e. fire treatment replicate) level prior to calculating ANPP. ANPP was determined for individual functional groups, both under mesquite canopies and in open grassland patches in each of the prescribed fire treatments. Whole community estimates of herbaceous ANPP were obtained by summing individual functional group ANPP values. This approach was used to capture small, but important periods of productivity which might otherwise be masked by functional groups whose productivity dynamics overlap in time. ANPP was calculated for each of the intervals that occurred between successive sampling periods, and these values were summed to obtain annual herbaceous ANPP estimates.

Statistical Analysis

Linear mixed model analysis, incorporating time as a repeated measure, was used to analyze productivity and biomass data with SPSS version 12.0.2 (SPSS 2004). Fire treatment, proximity to mesquite (sub-canopy vs. open grassland), and time following fire (year or month) were included as fixed effects. Replicate plots were nested within fire treatment and treated as a random effect, and a first order autoregressive (AR1)

covariance structure was employed to account for the use of repeated measures over time (Dai et al. 2006). Post hoc comparison of means was conducted using the Bonferroni procedure. P-values less than 0.05 were considered to be significantly different.

Results

Climate Relationships

Climate data collected over the course of the study, as well as long-term averages, are illustrated in Figure 1. Total precipitation in the first year of this study (May 2003 to May 2004) was 500.1 mm, which was approximately 25% lower than the long-term average of 665 mm. In the second year (May 2004 to May 2005), total precipitation was more similar to the long-term average, totaling 661.2 mm. Temperatures during the study approximated long-term averages.

Biomass Dynamics

Total Live Biomass

Total live biomass of the herbaceous community, quantified at approximately 6-week intervals, fluctuated seasonally (Figure 2 a,b,c) and differed significantly with respect to the interactions of fire treatment and location relative to mesquite canopy (hereafter, location); fire treatment and time; and location and time (Table 1). In both the summer fire treatment and unburned control, more herbaceous biomass occurred in open grassland patches than in areas under mesquite trees; however, the opposite was observed in the case of the winter fire treatment. Following winter fire treatment, greater levels of biomass were found under mesquite trees than in open grassland patches, thus underlying the statistically significant interaction observed between fire treatment and location (Table 1). The effects of both fire treatment and location were modified by their interaction with time. While herbaceous biomass fluctuated over the course of the study, seasonal peak in biomass tended to be greater following either winter or summer fire treatment than in the unburned control (Figure 2 a,b,c). Similarly, although total herbaceous biomass in open grassland patches was, on average, similar to

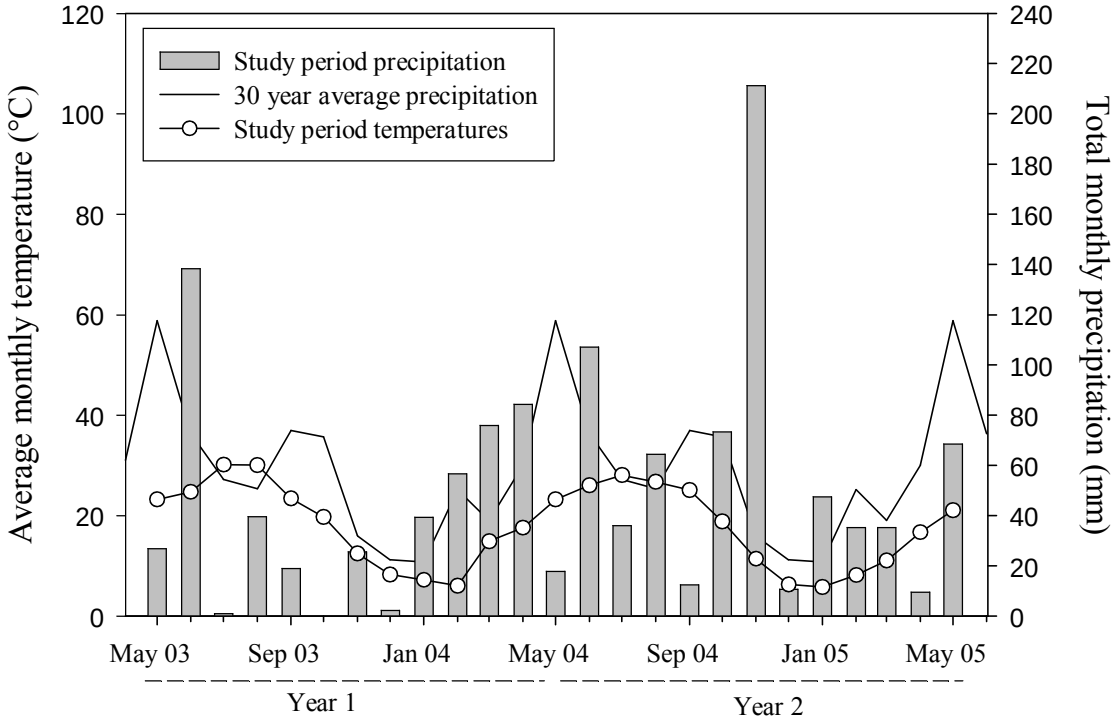

FIGURE 1. Monthly precipitation and temperature patterns for Vernon, TX. Average annual precipitation is 665 mm, and the average annual temperature is 16.1 °C. Over the course of this study, annual precipitation received in Year 1 (May 2003 to May 2004) equaled 500.1 mm, and total precipitation in Year 2 (May 2004 to May 2005) equaled 661.2 mm.

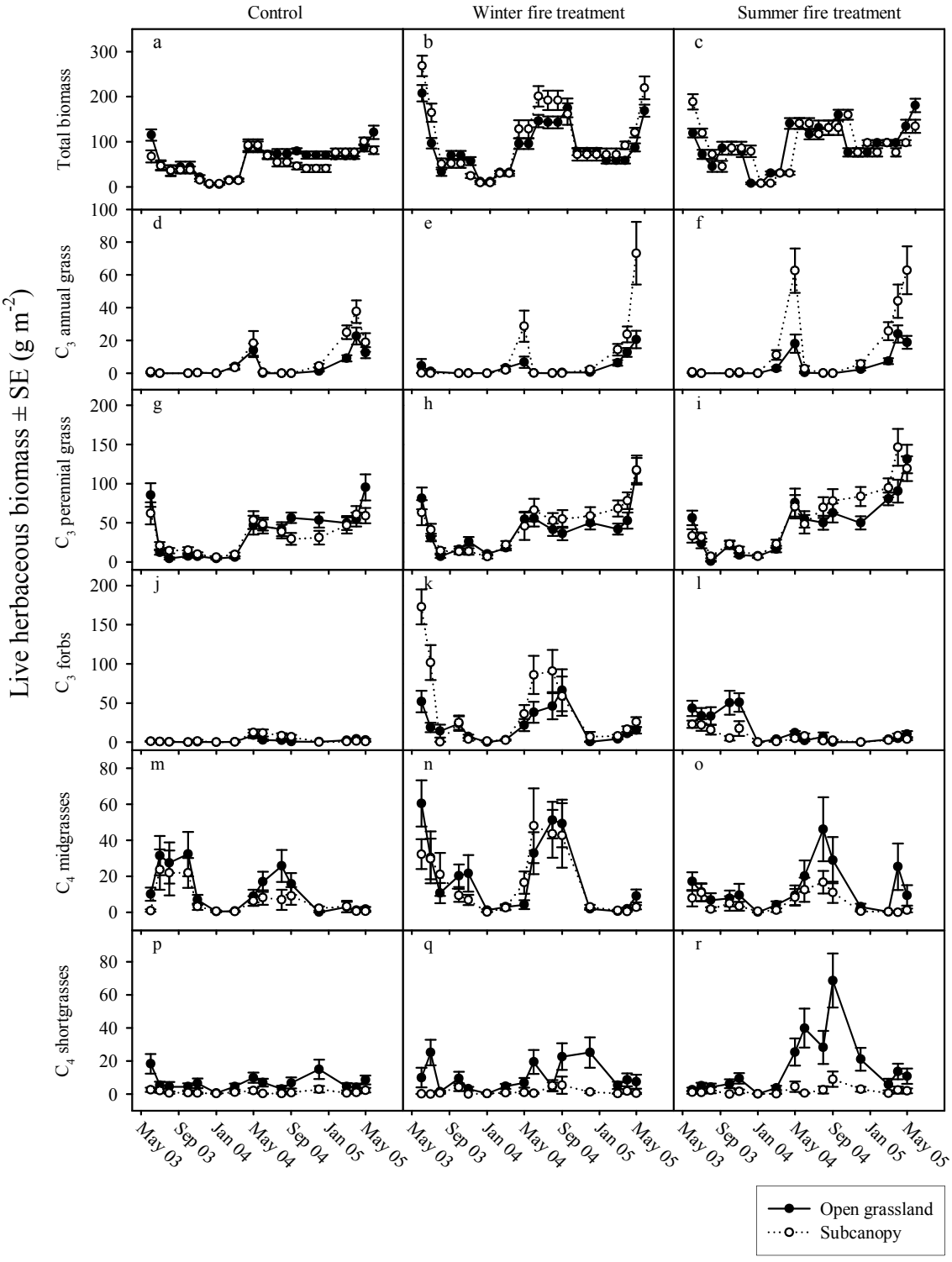

FIGURE 2. Average live biomass ($\bar{x}$ ± SE) of the total herbaceous community and its individual functional groups over the course of the study.

TABLE 1. Significance of the effects of fire treatment, time (i.e. month), location relative to mesquite canopy, and their interactions on live herbaceous biomass, as indicated by p-values. Total biomass represents the sum of each of the individual functional groups listed below.

Plant functional type	**Fire (F)**	**Location (L)**	**Time (T)**	**Interactions**			
				F*L	**F*T**	**L*T**	**F*L*T**
Total	< 0.001	0.911	< 0.001	0.008	< 0.001	0.021	0.076
C_3 annual grasses	0.120	0.002	< 0.001	0.264	< 0.001	< 0.001	0.268
C_3 perennial grasses	0.207	0.717	< 0.001	0.782	0.008	0.198	0.976
C_3 forbs	0.004	0.432	< 0.001	0.123	< 0.001	0.078	0.028
C_4 mid-grasses	0.020	0.073	< 0.001	0.705	< 0.001	0.245	0.959
C_4 short-grasses	0.311	0.003	< 0.001	0.424	< 0.001	< 0.001	0.007

or slightly greater than the herbaceous biomass under mesquite trees, temporal fluctuations in biomass occasionally led to more biomass occurring under mesquite.

C_3 Live Biomass: Annual and Perennial Grasses

C_3 annual grass live biomass dynamics differed significantly with respect to the interactions of fire treatment and time; and location and time (Table 1). C_3 annual grass biomass displayed pronounced peaks in the spring of each year, and this effect was enhanced, relative to the control, following either winter or summer fire treatment (Figure 2 d,e,f). Springtime peaks in C_3 annual grass live biomass also tended to be particularly enhanced under mesquite trees, as opposed to within open grassland areas.

C_3 perennial grass live biomass differed significantly with respect to an interaction between the effects of fire treatment and time, but not with canopy location (Table 1). Although C_3 perennial grass biomass tended to occur with similar abundance across all fire treatments and locations, sites that had been exposed to fire treatment supported somewhat greater amounts of biomass in the spring of 2005 (Figure 2 g,h,i).

C_3 Live Biomass: Forbs

C_3 forb live biomass differed significantly with respect to a three-way interaction among fire treatment, location, and time (Table 1). Following the winter fire treatment, C_3 forb biomass was greatest underneath mesquite and in the early part of the growing season (Figure 2k). In the summer fire plots, forb biomass occurred to a greater extent in open grassland areas and later in the growing season (Figure 2l). C_3 forb biomass was substantially less in the unburned control than in either of the fire treatments, but it did exhibit the same early season peak as the forbs in the winter fire treatment (Figure 2j).

C_4 Live Biomass: Mid- and Shortgrasses

C_4 midgrass live biomass differed significantly with respect to an interaction between fire treatment and time (Table 1). The C_4 midgrasses displayed distinct seasonal peaks in biomass, and these were greater extent in sites that had been exposed

to either winter or summer fire treatments (Figure 2 m,n,o). This response was greater following the winter fire treatment than the summer fire treatment; on average, winter fire plots supported 18.5 g biomass m^{-2}, while the summer fire plots supported an average of 9.4 g biomass m^{-2}.

C_4 midgrass biomass differed to a lesser extent with respect to location relative to mesquite canopy ($p = 0.073$); however, open grassland areas consistently supported greater C_4 midgrass biomass (15.0 g m^{-2}) than did subcanopy areas (9.9 g m^{-2}) when values were averaged across all fire treatments and sample dates.

C_4 shortgrass live biomass differed with respect to a significant three-way interaction of fire treatment, location, and time (Table 1). C_4 shortgrass biomass exhibited pronounced peaks in the mid-to-late growing season each year, and these peaks were enhanced in open grassland patches, following either of the seasonal fire treatments (Figure 2 p,q,r). Significantly greater amounts of C_4 shortgrass biomass occurred in open grassland areas (average, 11.2 g m^{-2}) than in subcanopy areas (average, 1.6 g m^{-2}), and biomass peaked to a greater extent following the summer fire treatment (Figure 2r) than in winter fire treatment sites (Figure 2q).

Total Standing Dead Biomass

Standing dead biomass dynamics of the total herbaceous community, quantified at approximately 6-week intervals, differed significantly with respect to the interactions of fire treatment and time, as well as fire treatment and location (Table 2). Seasonal peaks in standing dead biomass occurred in the late summer and late winter of each year, and they were more pronounced in the unburned control and winter fire treatment sites than following the summer fire treatment (Figure 3 a,b,c). On average, open grassland patches supported more standing dead biomass than did areas under mesquite (153.8 vs. 125.7 g biomass m^{-2}, respectively). The winter fire treatment contained the most standing dead biomass, while the relative ranking of the standing dead biomass found in the summer fire treatment and unburned control depended upon location. Under mesquite, the summer fire treatment supported more standing dead, on average, than did

TABLE 2. Significance of the effects of fire treatment, time (i.e. month), location relative to mesquite canopy, and their interactions on standing dead herbaceous biomass and litter, as indicated by p-values. Total biomass represents the sum of each of the individual functional groups listed below.

Plant functional type	**Fire (F)**	**Location (L)**	**Time (T)**	**Interactions**			
				F*L	**F*T**	**L*T**	**F*L*T**
Total	0.014	< 0.001	< 0.001	0.013	< 0.001	0.087	0.623
C_3 annual grasses	0.092	< 0.001	< 0.001	< 0.001	< 0.001	0.156	0.120
C_3 perennial grasses	0.526	0.001	< 0.001	0.055	< 0.001	0.131	0.980
C_3 forbs	0.034	0.002	< 0.001	< 0.001	< 0.001	0.129	0.580
C_4 mid-grasses	0.003	0.004	0.009	0.926	< 0.001	0.677	0.358
C_4 short-grasses	0.760	< 0.001	0.673	0.309	0.283	0.533	0.374
Total litter	0.003	< 0.001	< 0.001	0.001	0.000	0.071	0.212

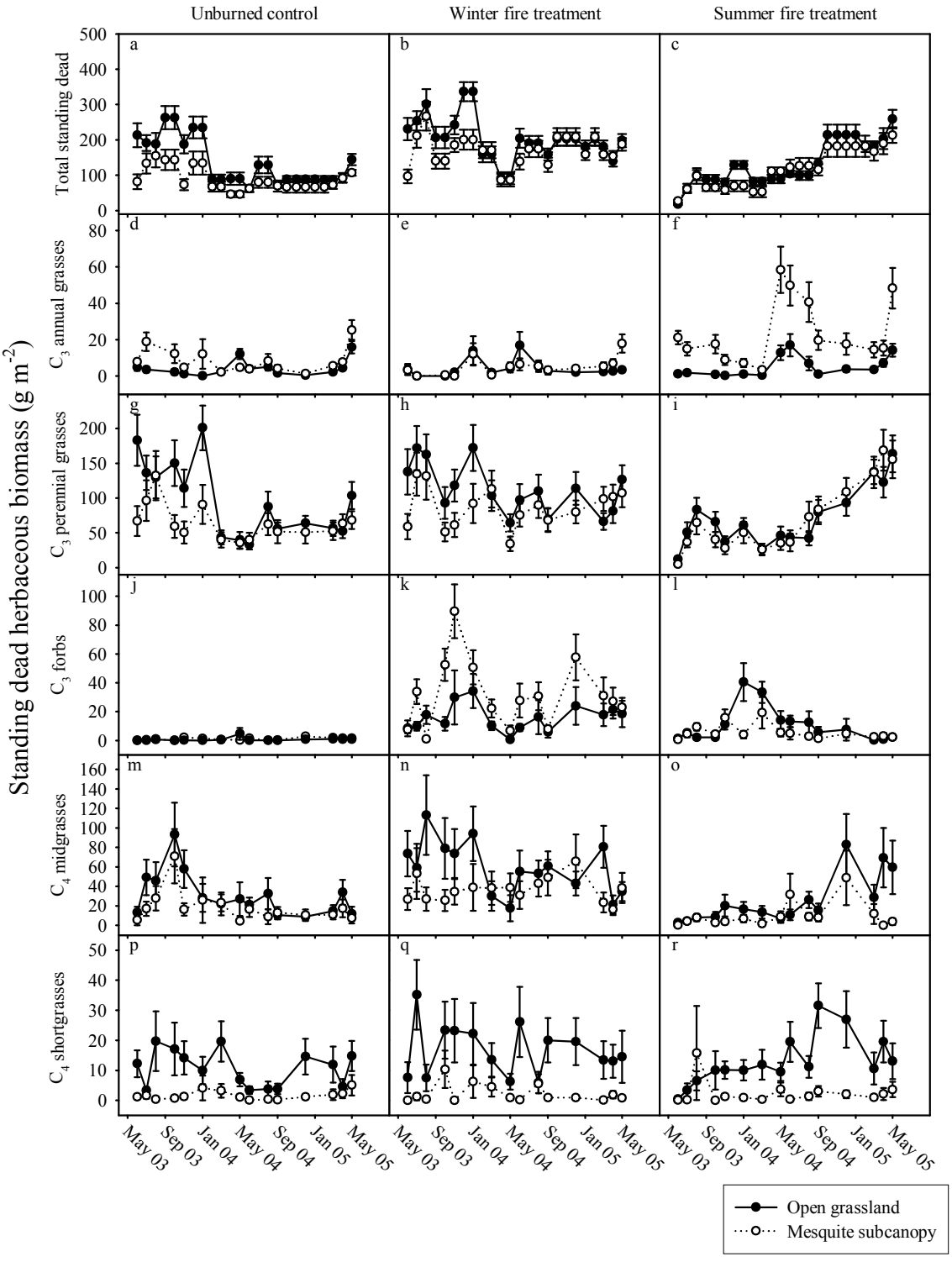

FIGURE 3. Average standing dead biomass ($\bar{x} \pm$ SE) of the total herbaceous community and its individual functional groups over the course of the study.

the unburned control (114.7 vs. 91.2 g biomass m^{-2}, respectively). Conversely, in open grassland patches, the unburned control supported more standing dead biomass, on average, than did the summer fire treatment sites (133.5 vs. 123.9 g biomass m^{-2}, respectively). Regardless of location, no significant differences were detected with respect to the amount of standing dead biomass occurring within the unburned control and summer fire treatments.

C_3 Standing Dead Biomass: Annual and Perennial Grasses

Similar to patterns observed with respect to its live biomass, C_3 annual grass standing dead biomass differed significantly with respect to the interactions of fire treatment and time; and location and time (Table 2). Seasonal peaks in C_3 annual grass standing dead biomass occurred from late spring to late summer and were enhanced by the summer fire treatment (Figure 3 d,e,f). At its peak, C_3 annual grass standing dead biomass tended to be greater under mesquite trees (average, 12.3 g m^{-2}) than in open grassland areas (average, 4.4 g m^{-2}). C_3 perennial grass standing dead biomass differed significantly with respect to the interaction of fire treatment and time, as well as the single effect of location (Table 2). Substantial fluctuation in C_3 perennial grass standing dead biomass occurred in both the unburned control and winter fire treatments over the course of the study (Figure 3 g,h). In the summer fire treatment, however, these fluctuations in time were less pronounced, and over the course of the last 6 months of the study, the C_3 perennial grass standing dead biomass showed a trend toward increasing biomass accumulation (Figure 3i). In addition, small, but significant, increases in C_3 perennial grass standing dead biomass were observed in open grassland patches (average, 89.2 g m^{-2}) relative to subcanopy areas (average, 72.3 g m^{-2}).

C_3 Standing Dead Biomass: Forbs

C_3 forb standing dead biomass differed with respect the interactions between fire treatment and location; and fire treatment and time. C_3 forb standing dead biomass was significantly greater in sites that had been exposed to winter fire (average, 25.9 g m^{-2})

than in unburned controls (average, 0.9 g m^{-2}) and tended to be greater under mesquite than in open grassland patches early in the growing season (Figure 3k). The summer fire treatment, however, supported greater amounts of standing dead forb biomass in the open grassland areas (Figure 3l).

C_4 Standing Dead Biomass: Mid- and Shortgrasses

C_4 midgrass standing dead biomass differed with respect to the interaction between fire treatment and time, as well as the single effect of location (Table 2). In the first year of the study, large wintertime peaks in C_4 midgrass standing dead biomass occurred in both the unburned control (Figure 3m) and winter fire treatment sites (Figure 3n), while occurring to a lesser degree in the summer fire treatment (Figure 3o). In the second year of the study, these peaks occurred in the winter and summer fire treatment sites, but not in the unburned control. Similar to patterns observed with respect to live biomass, open grassland areas consistently supported greater amounts of standing dead C_4 midgrass biomass than did subcanopy areas (Figure 3 m,n,o).

C_4 shortgrass standing dead biomass differed only with respect to location (Table 2). Significantly greater amounts of standing dead C_4 shortgrass biomass occurred in open grassland areas (average, 13.6 g m^{-2}) than in subcanopy areas (average, 2.3 g m^{-2}) (Figure 3 p,q,r).

Litter

Total litter mass (herbaceous plus mesquite-derived litter) differed significantly with respect to the interactions of fire treatment and location; and fire treatment and time (Table 2). On average, total litter mass was greatest in the unburned control (average, 433.6 g m^{-2}), intermediate following the winter fire treatment (average, 315.3 g m^{-2}), and least following the summer fire treatment (average, 226.8 g m^{-2}) (Figure 4 a,b,c). However, the ranking of these three treatments differed when location relative to mesquite was considered. In open grassland patches, the unburned control, winter fire,

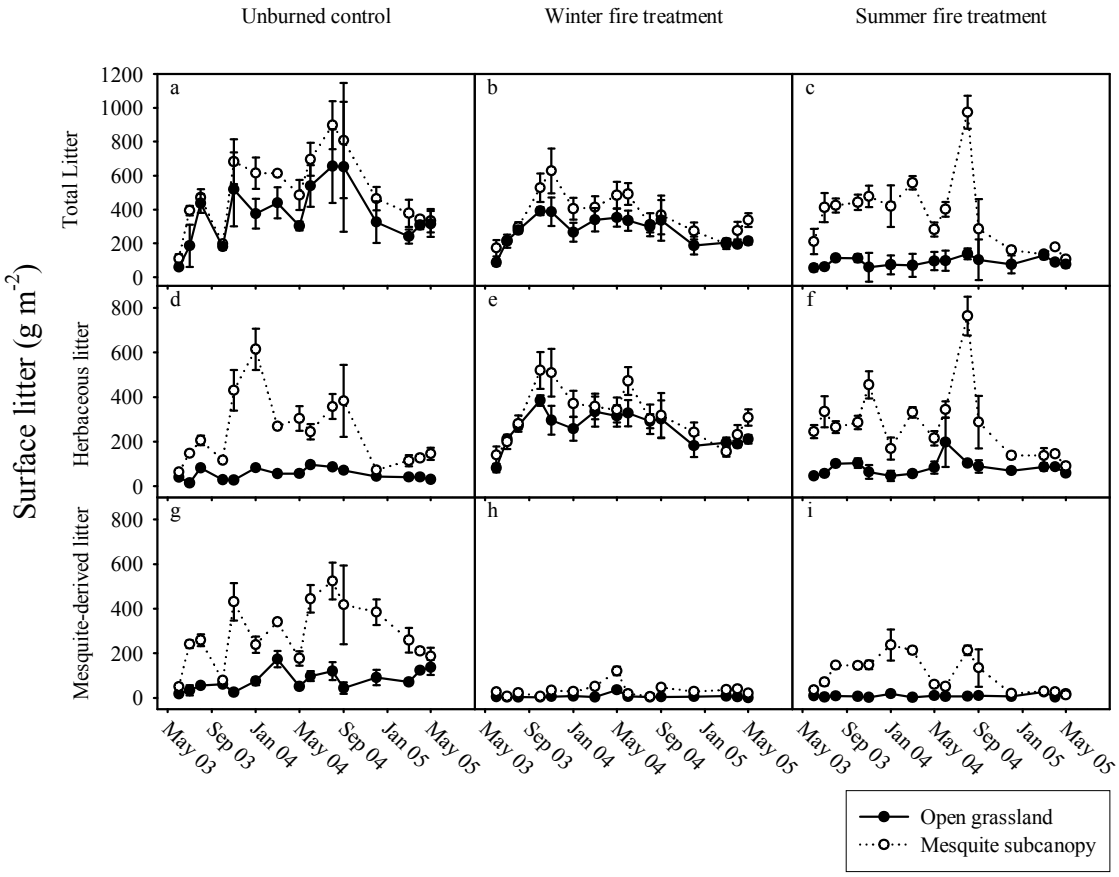

FIGURE 4. Total, mesquite-derived, and herbaceous layer litter dynamics. Symbols represent average surface litter masses ($\bar{x} \pm$ SE) over the course of the study.

and summer fire treatments supported average litter masses of 368.5, 271.6, and 89.6 g m^{-2}, respectively. Under mesquite, unburned controls supported the most total litter (average, 489.7 g m^{-2}) while the summer fire treatment supported slightly more total litter (average, 364.0 g m^{-2}) than the winter fire treatment (average, 358.9 g m^{-2}). As with other biomass pools examined in this study, litter mass values varied over time. Major peaks occurred in the late summer and autumn months, but the magnitude of these peaks varied with fire treatment (Figure 4 a,b,c). Large peaks in litter mass occurred at the end of the 2004 growing season in both the unburned control and summer fire treatment but were absent from the winter fire treatment.

ANPP

Total ANPP

The ANPP of the total herbaceous community differed significantly with respect to fire treatment. Sites exposed to winter fires were significantly more productive than those that had been exposed to summer fires or were left unburned. The ANPP in the summer fire treatment was greater than, but not statistically different from, the unburned control (Table 3). Total ANPP values following winter fire treatment averaged 434 g m^{-2} y^{-1}, ranging from 384 to 510 g m^{-2} y^{-1}, and ANPP following summer fire treatment averaged 313 g m^{-2} y^{-1} (range 270 to 355 g m^{-2} y^{-1}). In contrast, ANPP from the unburned control averaged 238 g m^{-2} y^{-1} over the two years of the study and ranged from 188 to 312 g m^{-2} y^{-1}. No significant differences among ANPP rates were found with respect to the effects of time following fire (i.e. year), location, or interactions among fire treatment, time, and location (Figure 5 a,b).

C_3 ANPP: Annual and Perennial Grasses

C_3 annual grasses comprised 4 to 31% of total ANPP in this system and their rates of ANPP differed significantly with the individual effects of time and location (Table 3). C_3 annual grasses were more productive under mesquite canopies and in the second year of the study (Figure 5 c,d). No significant differences in the ANPP of C_3

TABLE 3. Significance of the effects of fire treatment, time (i.e. year of study), location relative to mesquite canopy, and their interactions on annual herbaceous ANPP, as indicated by p-values. ANPP was calculated as the sum of increments of live and standing dead herbaceous biomass, and total ANPP represents the sum of each of the individual functional groups listed below.

Plant functional type	**Fire (F)**	**Location (L)**	**Year (Y)**	**Interactions**			
				F*L	**F*Y**	**L*Y**	**F*L*Y**
Total ANPP	0.005	0.278	0.156	0.177	0.116	0.583	0.317
C_3 annual grasses	0.670	0.002	0.038	0.554	0.649	0.360	0.592
C_3 perennial grasses	0.572	0.817	0.299	0.467	0.511	0.997	0.218
C_3 forbs	0.015	0.036	0.010	0.008	0.338	0.403	0.024
C_4 mid-grasses	0.385	0.015	0.239	0.127	0.008	0.216	0.324
C_4 short-grasses	0.424	0.010	0.903	0.358	0.068	0.591	0.043

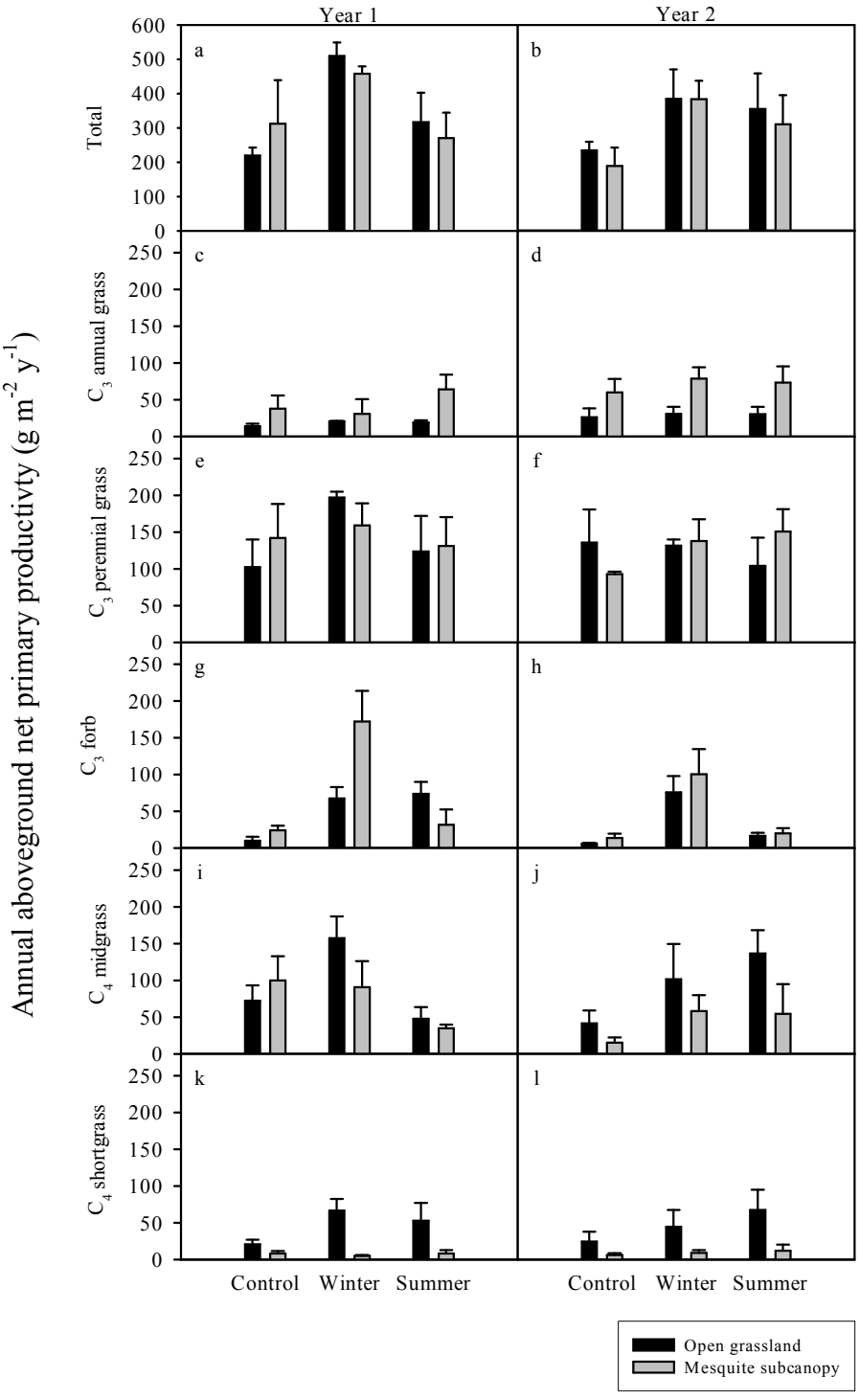

FIGURE 5. Average total annual herbaceous ANPP ($\bar{x} \pm$ SE). Total annual ANPP was calculated as the sum of increments of live and standing dead herbaceous biomass over time.

annual grasses were found with respect to fire treatment or interactions among fire treatment, time, or canopy cover.

The ANPP of C_3 perennial grasses comprised 26 to 58% of total ANPP but did not differ significantly with respect to the effects of fire treatment, time, location, or their interactions (Figure 5 e,f).

C_3 ANPP: Forbs

C_3 forbs comprised 2.5 to 38% of total ANPP and differed significantly with respect to the three-way interaction among fire treatment, location, and time (Table 3). Sites that were exposed to winter fire treatment sustained greater levels of forb ANPP than unburned controls or sites that had been exposed to summer fire treatment. Forbs were significantly more productive in subcanopy areas than in open grassland patches, and forbs were also more productive in the first year of the study than in the second year (Figure 5 g,h). The interaction observed between fire treatment, time, and location was the result of decreased levels of forb productivity in the second year of the study at all locations and under all fire treatments, except in open grassland patches that had been subject to the winter fire treatment. In these cases, productivity trends were opposite and displayed a slight increase from 68 g m^{-2} y^{-1} to 75 g m^{-2} y^{-1}.

C_4 ANPP: Mid- and Shortgrasses

C_4 midgrass productivity comprised 8 to 38% of total ANPP and differed significantly with respect to location and an interaction between the effects of fire treatment and year (Table 3). C_4 midgrasses were significantly more productive in open grassland patches than under mesquite (Table 3). The interaction observed between fire treatment and year occurred as a result of C_4 midgrass productivity increasing in the second year following summer fire treatment, while decreasing in both the unburned control and the winter fire treatments during that same period of time (Figure 5 i,j).

The productivity of C_4 shortgrasses comprised 3 to 19% of total ANPP and differed significantly with respect to the interaction of fire treatment, location, and year

(Table 3). C_4 shortgrasses were consistently and significantly more productive in open grassland patches than under the canopy of mesquite. The interactive effects of fire, location, and year were the result of decreased C_4 shortgrass productivity from the first year of the study to the second, in subcanopy areas of the unburned controls and open grassland patches of the winter fire treatment sites (Figure 5 k,l). At all other sites, C_4 shortgrass productivity increased.

Discussion

Net primary productivity varies considerably in natural systems and is known to be influenced by a number of biotic and abiotic controls, including climate, soils, plant community composition, and disturbance regime, in addition to other natural and anthropogenic factors (Field et al. 1995, Burke et al. 1997, Sala 2001). The results of this study demonstrate this variability, as well as the potential for disturbances, like woody plant encroachment and fire, to influence community structure and productivity rates significantly. Rates of herbaceous ANPP were found to range from 188 to 510 g dry matter m^{-2} y^{-1} across all treatments and years in this mesquite-encroached mixed grass savanna ecosystem, and community composition was found to vary with the presence or absence of mesquite canopy.

Previously published estimates of productivity for the southern mixed grass prairie region are similar to those quantified here, with values ranging from 157 to 450 g dry matter m^{-2} y^{-1} (Koos et al. 1962, Heitschmidt et al. 1986, Sala et al. 1988, Imhoff et al. 2004). The degree to which these values account for local scale variation is uncertain, as climate variation and changes in management may result in productivity rates that are much lower (e.g. 30 to 130 g dry matter m^{-2} y^{-1} [Ansley et al. 2004]). While the productivity estimates presented above represent more than a three-fold range in values, it is thought that the differences existing among them are due, in large part, to the methods used and the data sets upon which the models were based (Scurlock et al. 2002), rather than as a result of ground-level variability. It is thought that many regional estimates of ANPP for grassland systems suffer from the effects of homogenization

(Lauenroth 1979) and fail to account for the differences in productivity that may result from local scale variability. While homogenization of rates is inevitable as quantification is scaled up from to regional and global processes or phenomena, the recognition of variation in process rates at local levels is important, too, particularly in light of attempts to understand and model potential trajectories of communities and ecosystems undergoing change.

Stimulation by Fire

As hypothesized, sites that were exposed to prescribed fire treatments supported greater rates of herbaceous ANPP than did those that remained unburned. Pooled across both years of the study and locations relative to mesquite, rates of ANPP quantified following winter and summer fire treatments averaged 434 and 313 g dry matter m^{-2} y^{-1}, respectively, while those from the unburned control averaged 238 g dry matter m^{-2} y^{-1}. At the level of the individual plant functional groups, the C_3 forbs were the only group observed to experience a statistically significant change in productivity rate following fire treatment. However, this increase coupled with the additive effects of positive, but non-significant, shifts in ANPP across most or all of the other functional groups in response to fire resulted in the emergence of significant differences in productivity at the whole community level (Table 3).

Fire's ability to stimulate growth and productivity in grassland ecosystems is often linked to the removal of surface litter (Daubenmire 1968, Wright and Bailey 1982, Knapp and Seastedt 1986, Henry et al. 2006). Surface litter removal has been observed to result in greater light availability, warmer soil temperatures, more active soil microbial communities, and enhanced rates of nitrogen mineralization (Knapp and Seastedt 1986, Blair 1997). In this study, surface litter masses decreased following fire treatment, and soil microbial biomass increased by ~20% relative to the unburned control (Hollister et al., unpublished data). Microbial biomass (as inferred by soil microbial biomass C and N) and gross nitrogen mineralization rates have been shown to share a significant, positive relationship with one another (Booth et al. 2005). Although

direct evidence for enhanced nitrogen mineralization has been difficult to detect in this system (Harris 2005), the increases in soil microbial biomass that have been observed suggest that nitrogen mineralization occurs at increased rates following fire treatment and may be available in greater quantities to support NPP.

While the broad stimulation of community wide ANPP following both winter and summer fire treatments may appear to contradict commonly held assumptions regarding the effects of fire, it should be recognized that much of the knowledge regarding the effects of prescribed fire in mixed grass systems, to date, has been based upon assessments of individual species' responses to fire treatment (Launchbaugh 1964, Wright 1974). Such a knowledge base makes the prediction of community level behavior challenging given that: a) the dynamics of individual species may not necessarily be representative of the community as a whole; b) reports regarding the nature of individual species' responses to fire are often conflicting (as summarized by Wright 1974); and c) point-in-time assessments of species' responses, such as peak- or end-of-season biomass, may not adequately characterize their post-fire behavior. A recent evaluation of prescribed fire literature from the central Great Plains (Engle and Bidwell 2001) concluded that the region's native grasslands may be more resilient to fire, in any season, than has previously been acknowledged.

Fire Effects on Community Composition

Although it is commonly assumed that community composition will be altered as a function of the season in which fire occurs, the amount of evidence that exists to support such changes is rather limited (Engle and Bultsma 1984, Howe 1994, 1995, Engle and Bidwell 2001) and their functional significance is rarely evaluated. In this study, potential fire-induced changes in community composition were assessed in terms of end of season biomass, as well as functional group contribution to total annual ANPP. The results of this study suggest that the detection and interpretation of community response to fire treatment depend upon the techniques that are used and the temporal context within which they are applied.

From the perspective of functional group contributions to total annual ANPP, few consistent or significant differences in the relative contributions of each of the functional groups to total ANPP were found following repeated seasonal fire treatments (Table 4). Across all fire treatments, C_3 annual grasses accounted for 10 to 15% of ANPP, C_3 perennial grasses accounted for 36 to 50% of ANPP, C_4 midgrasses accounted for 21 to 23% of ANPP, and C_4 shortgrasses accounted for 7 to 10% of ANPP. C_3 forbs were the only functional group that showed a significant increase in productivity in response to fire treatment. Forb productivity, relative to total ANPP, was significantly greater at sites that were exposed to winter fire treatment than in sites that had been exposed to summer fire treatment or were left unburned. Forbs accounted for an average of 24% of total ANPP in winter fire sites, as opposed to 11% of total ANPP following summer fires and 6% of total ANPP in the unburned control.

In contrast, the use of end of season data provided quite different results, lending support to the idea that seasonal fire treatments will alter the C_3/C_4 balance of mixed grass assemblages. Previous studies that have documented shifts in community composition as a function of seasonal fire treatment have used post-fire, end-of-growing-season data describing the incidence, biomass, and/or percent cover of major species or functional groups to demonstrate these changes (Anderson et al. 1970, Engle and Bultsma 1984, Howe 1994, 1995). Using an end-of-growing-season biomass approach to characterize community composition in this study, trends that paralleled predictions of altered composition began to emerge.

Observations of total standing biomass at the end of the 2004 growing season (September 2004) from this study would suggest that winter fires enhanced the production of C_4 midgrass species (110.15 g m^{-2} vs. 26.58 g m^{-2}) and forbs (72.07 g m^{-2} vs. 0.88 g m^{-2}) relative to the unburned control, while summer fires similarly enhanced the production of C_3 perennial grasses relative to the unburned control (144.84 g m^{-2} vs. 112.27 g m^{-2}). C_4 shortgrass end of season biomass was 4 to 10 times greater than that

TABLE 4. Significance of the effects of fire treatment, time (i.e. year of study), location relative to mesquite canopy, and their interactions on relative ANPP, as indicated by p-values. ANPP was calculated as the sum of increments of live and standing dead herbaceous biomass, and relative ANPP represents contribution of each functional group to total ANPP.

Plant functional type	**Fire (F)**	**Location (L)**	**Year (Y)**	**Interactions**			
				F*L	**F*Y**	**L*Y**	**F*L*Y**
C_3 annual grasses	0.262	0.005	0.004	0.528	0.121	0.162	0.245
C_3 perennial grasses	0.306	0.576	0.650	0.473	0.275	0.532	0.712
C_3 forbs	0.004	0.257	0.023	0.110	0.122	0.725	0.011
C_4 mid-grasses	0.885	0.040	0.226	0.897	0.005	0.187	0.398
C_4 short-grasses	0.527	0.014	0.529	0.725	0.963	0.840	0.851

of the unburned control (5.70 g m^{-2}), increasing after both winter (24.42 g m^{-2}) and summer (56.06 g m^{-2}) fire treatments. Although the C_4 shortgrass results are somewhat incongruent with the compositional shifts that might be expected as a result of seasonal fire treatment, recent work has shown that buffalograss, the most abundant C_4 shortgrass at this site, responds positively to both winter and summer fire treatments (Ansley and Castellano 2007).

Why do the interpretations of fire effects differ when ANPP is considered instead of end-of-season biomass, or vice versa? Although ANPP is a function of community composition, the two are not necessarily synonymous. Composition is indicative of community structure at a given point in time, while ANPP reflects community function over longer time scales, incorporating the dynamics of growth, senescence, and decomposition. Mixed grass prairie ecosystems typically experience multiple peaks in productivity, as well as declines in biomass, throughout the year (Figure 6). Given the dynamic nature of these systems, it is unlikely that either measure will fully capture the details of the other. Thus, it is important to consider aspects of both structure and function as we seek to understand ecosystems and the processes that serve to alter them.

Mesquite Drives Alternate Dynamics

Studies in savanna ecosystems around the world have demonstrated that tree-grass interactions vary widely (Belsky et al. 1989, Weltzin and Coughenour 1990, Mordelet and Menaut 1995, Scholes and Archer 1997, Reich et al. 2001) and that the nature of such interactions depends upon individual species' resource requirements and ecophysiology; resource availability; and disturbance regime (Scholes and Archer 1997). Mesquite is often regarded as having negative impacts on its associated herbaceous community, but the degree to which those effects are suppressive may be over-generalized. While greater densities of mesquite have been shown to limit herbaceous growth and productivity (Ansley et al. 2004), other evidence suggests that light densities (< 25 to 30% canopy cover) may be beneficial (Heitschmidt et al. 1986, Ansley and Castellano 2006).

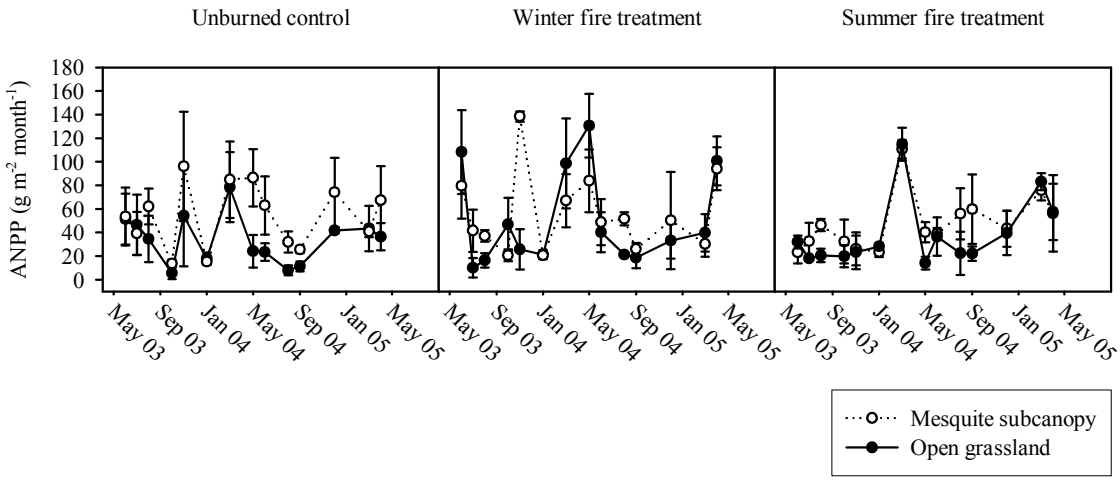

FIGURE 6. Total herbaceous ANPP fluctuates over time and experiences multiple peaks over the course of each year. Symbols represent average ANPP rates ($\bar{x}$ ± SE) between sampling times over the course of the study.

Whether suppressive or facilitative, the effects of mesquite on the understory herbaceous community may be attributed to its ability to act as an ecosystem engineer, altering the flow, distribution, and availability of resources within its microenvironment (Gutierrez and Jones 2006). Mesquite is a known nitrogen fixer (Johnson and Mayeux 1990, Zitzer et al. 1996), and its presence in grassland ecosystems often leads to the establishment of nutrient-enriched zones within the grassland matrix (Tiedeman and Klemmedson 1973). Mesquite also has the potential to redistribute moisture from deep in the soil profile to surface soil layers (i.e. conduct hydraulic lift) (Zou et al. 2005), and its canopy may moderate solar radiation and soil temperatures, particularly during the peak of the summer (Tiedemann and Klemmedson 1977).

The nutrient enrichment effects associated with mesquite persist in the soil even after individual trees have been damaged or killed (Biggs et al. 2002, Dai et al. 2006). While fire treatment generally does not kill mature mesquite (Ansley and Jacoby 1998), it does allow a window of opportunity to assess the effects of soil nutrient enrichment in the absence of competition for light. During the period of time in which mesquite is resprouting and recovering from disturbance, it might be expected that the combination of nutrient enriched soils and release from competition for light would allow the herbaceous community in former subcanopy areas to flourish. Thus, it was hypothesized that soil nutrient enrichment and the canopy cover have interactive effects on herbaceous ANPP, leading to lower ANPP rates in subcanopy areas of the unburned controls where competition for light remained intact, and increased herbaceous ANPP in former "subcanopy" areas where the canopy had been removed by fire but nutrient enrichment effects remained.

The results of this study suggest that significant interactions between the effects of soil nutrient enrichment and canopy cover may influence total "subcanopy" ANPP (Table 5). Following fire treatment in either winter or summer, rates of total "subcanopy" ANPP tended to be greater than corresponding rates observed in the unburned controls (Table 5). This difference was significant only in the comparison of the winter fire treatment versus the unburned control, and it was driven almost entirely

TABLE 5. Herbaceous ANPP in the mesquite subcanopy and former subcanopy areas. ANPP was calculated as the *sum of increments of live and standing dead biomass*, and the values displayed here represent annual ANPP averaged over the two years of the study. Total ANPP represents the summed ANPP of each of the five grass and forb functional groups listed below. Graminoid ANPP represents the total ANPP of the grass functional groups only.

Fire treatment	**Subcanopy ANPP (g m^{-2} y^{-1})**						
	Total	**Gram.**	**C_3A**	**C_3P**	**C_3F**	**C_4M**	**C_4S**
Unburned	250.2 [a]	231.1	49.1	117.4	19.1 [a]	57.4	7.3
Winter fire	420.8 [b]	284.5	54.8	148.3	136.3 [b]	74.3	7.2
Summer fire	290.4 [a]	264.6	68.8	141.0	25.8 [a]	44.6	10.2

*Gram. = graminoid, C_3A = C_3 annual grasses, C_3P = C_3 perennial grasses, C_3F = C_3 forbs, C_4M = C_4 midgrasses, C_4S = C_4 shortgrasses. Values within a column, followed by the same letter or no letter, are not significantly different from one another ($p < 0.05$).

by differences in the productivity of the C_3 forb functional group (Figure 5, C_3 forbs ANPP). The C_3 forbs were the only functional group in which a significant interaction between the effects of fire treatment and mesquite canopy on ANPP was detected (Table 3). When the forbs were removed from the analysis of total ANPP, no significant differences remained.

While the interaction between fire treatment and mesquite was not as strong as initially hypothesized, the more striking result that does emerge from this analysis is the impact of mesquite alone on the structure and function of the surrounding herbaceous community. All of the functional groups analyzed, with the exception of the C_3 perennial grasses, showed significant differences in ANPP with respect to their location relative to the mesquite canopy (Table 3). C_4 midgrass and shortgrass species were significantly more productive in open grassland patches than in subcanopy areas, while the C_3 annual grasses and forbs were significantly more productive under mesquite canopies than in open grassland patches.

Competition for light has been noted as an important factor contributing to the reduced yields of C_4 grasses often observed in the presence of mesquite (Ansley et al. 2004). Other factors that may serve to limit the persistence, productivity, and distribution of the C_4 grasses include the accumulation of surface litter (Knapp and Seastedt 1986) and altered soil nitrogen availability (Tilman and Wedin 1991). In contrast, these same mechanisms appear to promote the establishment and productivity of C_3 annual grass species: 1) C_3 annuals are unlikely to be affected by competition for light, as they tend to complete their life cycle before the emergence of the mesquite canopy in the spring (Ansley et al. 2004); 2) C_3 annual grasses, including *Bromus japonicus* Thunb. *ex* Murray (Japanese brome), one of the most abundant C_3 annuals at this site, often benefit from the accumulation of surface litter, as it provides conditions that are well suited to the germination and establishment of annual species (Whisenant and Uresk 1990); and 3) C_3 grass species may be poor competitors for resources in nutrient-poor soil (Tilman and Wedin 1991), and thus, would benefit from the increased stores of nitrogen that typically occur in the soils beneath mesquite.

Through its ability to alter resource distribution and abundance, mesquite appears to have also altered the distribution and abundance of functional groups within the herbaceous community. While this shift has not affected total ANPP under mesquite versus that of open grassland patches, the changes that have occurred with respect to community composition have the potential to other aspects of ecosystem function. Changes in species composition may lead to altered patterns of nutrient cycling (Hobbie 1992) and trophic interaction, particularly as plant tissue chemistry, rooting patterns, and phenology may shift as a result of such changes. Given the extent of grassland ecosystems and the common occurrence of woody plant encroachment worldwide, such changes have the potential to impact global nutrient cycles.

Conclusions

Land use and land cover change play an important role in shaping ecosystem patterns and processes. The results of this study demonstrate that both fire treatment and woody plant encroachment have the potential to alter the structure and function of a southern mixed grass savanna ecosystem. Repeated seasonal fire treatments led to increased rates of ANPP, relative to that of the unburned control, and altered community composition, but fire treatment did not alter the relative contribution of individual functional groups to total ANPP. With respect to the effects of mesquite, ANPP rates did not differ significantly between open grassland patches and mesquite subcanopy areas, but community composition did. Open grassland areas supported greater proportions of C_4 grass species, but a lesser proportion of C_3 annual grasses, than did mesquite subcanopy areas. The results of this study also demonstrate that the perspective from which we study ecosystem dynamics, and the techniques we use to do so, may be very important to the ways in which treatment outcomes are perceived. The detection and interpretation of changes to both ecosystem structure and function have important implications for the ways in which we assign value to lands and make decisions regarding their use and management, as well as for our ability to accurately model and predict outcomes in the future.

CHAPTER III

EFFECTS OF PRESCRIBED FIRE AND WOODY ENCROACHMENT ON SOIL ORGANIC CARBON POOL SIZES AND TURNOVER RATES

Introduction

Soil organic carbon (SOC) is composed of a continuum of carbon-based compounds that vary in chemical complexity, age, and origin. The storage of organic carbon in the soil occurs as a result of the net balance between organic matter inputs to and decompositional losses from the soil system (Schlesinger 1997, Amundson 2001). As such, SOC concentrations are sensitive to, and have the potential to reflect, shifts in plant community composition, land use, and management regimes, as well as changing climate (Schlesinger 1997, Collins et al. 2000, McLauchlan and Hobbie 2004, Cheng et al. 2007). The degree to which land use and land cover changes may alter SOC concentrations is of both interest and concern with respect to the global carbon cycles, as some land use and land cover changes may serve to sequester atmospheric carbon (Follett et al. 2001, Lal 2004), while others have the potential to promote carbon loss back to the atmosphere (Schlesinger 1997).

Over the course of the last century, dramatic increases in the density and distribution of *Prosopis glandulosa* Torr. (honey mesquite) have occurred in the mixed grass prairies of the southern Great Plains, USA (Van Auken 2000, Ansley et al. 2002, Asner et al. 2003). The encroachment of this nitrogen-fixing, woody species into grassland ecosystems often results in increased levels of above- and belowground plant biomass, as well as enhanced stores of carbon and nitrogen in plants and soils (Archer et al. 2001, Hibbard et al. 2001, Liao et al. 2006b). Despite the potential gains in carbon storage that may occur as the result of mesquite encroachment, this land cover change is often associated with the loss of native grassland species and reductions in the productivity and economic viability of rangeland livestock production (Archer et al. 2001). Seeking to control or slow the spread of mesquite, landowners often employ mechanical removal methods and/ or herbicide treatment in the management of

mesquite-encroached rangelands, but the use of prescribed fire has begun to emerge as a popular, lower-cost alternative (Archer et al. 2001, Teague et al. 2001). Fire tends to reduce the standing biomass of woody plants and other invasive species while having a stimulatory effect on the productivity of native grasses (Wright and Bailey 1982, Collins and Wallace 1990).

Despite the common occurrence of mesquite encroachment as a land cover change phenomenon and the increasingly common use of fire as a land management tool, we lack substantial quantitative information concerning both their individual and combined abilities to influence the SOC dynamics and the carbon storage potential of rangeland ecosystems. It has been shown that the woody encroachment of grassland systems often leads to increased accumulation of organic carbon in the soil (San Jose et al. 1998, Tilman et al. 2000, Pacala et al. 2001, Liao et al. 2006b, Zavaleta and Kettley 2006). Differences in productivity, tissue chemistry, and decomposability are frequently cited as factors which may contribute to such altered SOC concentrations (Enriquez et al. 1993, Hobbie and Chapin 1996, McCulley et al. 2004, Liao et al. 2006a). The accretion of SOC following woody plant encroachment, however, does not appear to be universal. Other studies suggest that woody plant encroachment may cause no net change in SOC (McCarron et al. 2003, Smith and Johnson 2003, Hughes et al. 2006) or that it may even lead to ecosystem carbon loss (Jackson et al. 2002, Hudak et al. 2003).

The effects of fire on carbon cycling and SOC storage have also been shown to vary widely. While some studies suggest that prescribed fire may lead to increased SOC storage (Ansley et al. 2006a, Dai et al. 2006), others have reported no net change (Rice and Owensby 2000, Roscoe et al. 2000), or negative effects on SOC storage (San Jose et al. 1998, Bird et al. 2000) following fire treatment. Although biomass burning represents an abrupt disruption to flow of carbon inputs from plants and litter to soils, the occurrence of fire in temperate grassland systems often results in the stimulation of net primary productivity and leads to enhanced levels of biomass accumulation (Knapp and Seastedt 1986, Blair 1997, Ansley et al. 2002). Such gains are thought to offset combustion-related carbon loss and, potentially, contribute to increased SOC storage

(Rice and Owensby 2000, Ansley et al. 2002). Conversely, the removal of biomass and litter, as well as the deposition of ash and char, may alter the soil physical environment and lead to increased soil temperatures and soil microbial activity (Tiedemann and Klemmedson 1986, Ojima et al. 1994, Knapp et al. 1998), stimulating loss of carbon from the soil. Previous research at this site suggests that, in some cases, prescribed fire treatment may lead to increased SOC storage under *Nassella leucotricha* (Trin. & Rupr.) Pohl (Texas wintergrass), a native C_3 grass species (Ansley et al. 2006a). However, the chemical nature and stability of this additional SOC, as well as the applicability of these results to other plant species or functional groups is currently unknown.

Studies of SOC often simplify its complex continuum of compounds into three operationally defined pools characterized by their chemical recalcitrance and mean residence times (Jenkinson and Rayner 1977, Parton et al. 1987, Paul et al. 2006). The active, or labile, SOC pool is composed of recent organic inputs that cycle rapidly and decay on the order of days to weeks. The slow, or intermediate, turnover SOC pool is composed of more complex and slowly decaying materials that typically turnover on the order of years to decades. The resistant, or recalcitrant, SOC pool is thought to be composed of complex compounds, which may be physically or chemically protected by the soil matrix, and are thought to decay on the order of centuries to millennia. A combination of long-term incubation, acid hydrolysis, and kinetic modeling are typically used to quantify these operationally distinct pools (Collins et al. 2000, Paul et al. 2006).

The objective of this study was to quantify the effects of fire and woody plant encroachment on the SOC dynamics of a savanna-like, mesquite-encroached mixed grass prairie ecosystem in the southern Great Plains. To accomplish this, pool sizes and turnover rates of the active, slow-turnover, and resistant forms of SOC in surface soils (0 to 10 cm) were quantified relative to vegetation type (mesquite, C_3 grasses, and C_4 grasses) and fire treatment (unburned, winter fire treatment, summer fire treatment) using a combination of long-term incubation, acid hydrolysis, and kinetic modeling techniques (Collins et al. 2000, Paul et al. 2006). We hypothesized that a) more SOC would be stored in soils occurring under mesquite than in those occurring under

grassland patches, regardless of fire treatment; b) the SOC occurring under mesquite would contain a greater proportion of slow-turnover and resistant carbon than that of the grassland; and c) fire-induced increases in the net primary productivity of grassland patches would lead to concomitant increases in SOC storage.

Materials and Methods

Study Site

This study was conducted in a mesquite-encroached southern Great Plains mixed grass savanna system, located near Vernon, TX (33°51'20" N, 99°26'50" W). Mean annual precipitation at this site is 665 mm, which is bimodally distributed with peaks in May and September. The mean annual temperature is 16.1^{o} C, and monthly average extremes range from 36^{o} C in mid-summer to -2.5^{o} C in mid-winter (Ansley et al. 1990). The soils at this site are classified as fine, mixed, superactive, thermic Vertic Paleustolls of the Tillman series, with 0 to 1% slope (Soil Survey Staff 2007), and the surface soils (0 to10 cm) contain a particle size distribution of 32% sand, 52% silt, and 16% clay (Dai et al. 2006).

The plant community is comprised of a mixture of C_3 grass species, C_4 grass species, and forbs in the herbaceous layer, and the overstory is dominated by mesquite. Texas wintergrass is the dominant C_3 grass species, and dominant C_4 grasses include the midgrasses *Panicum obtusum* Kunth (vine mesquite) and *Sporobolus compositus* (Poir.) Merr. (meadow dropseed), as well as the stoloniferous shortgrass, *Buchlöe dactyloides* Nutt.

The experimental plots in which this study was conducted were established in 1990 to study ecosystem response to prescribed fire. The fire treatments examined in this study included repeated summer fires (burned during the months of August to September in 1992, 1994, and 2002), repeated winter fires (burned during the months of January to March in 1991, 1993, 1995, and 2002), and a series of unburned controls. Fires were conducted as headfires and were supported by fine fuel loads ranging from 1300 to 4285 kg ha^{-1} (Ansley et al. 2006a, Dai et al. 2006). Each treatment consisted of

3 replicate plots, ranging from 1 to 6 ha in size. Grazing has been excluded from the sites since 1988, and fire records indicate that fire has been excluded from the unburned control sites since the 1960s (Ansley et al. 2006a).

Soil Collection and Processing

In each of the 3 replicated fire treatments, two soil samples were collected beneath each of three major vegetation types (mesquite, C_3 grass, and C_4 grass) in October 2003. Each of these two soil samples was a composite of 4 cores, each of which was taken beneath a different plant. Surface litter was gently brushed away and cores of mineral soil (2.5 cm diameter x 10 cm depth) were collected at the base of mesquite trees and under the crowns of C_3 grasses and C_4 grasses. A subsample of each soil composite was used to determine gravimetric water content and bulk density by drying at 105 °C for 24 hours. The remainder of each soil sample was stored at 4 °C until further analysis.

Long-term Incubation

A subsample of each field-moist soil composite was sieved through a 4 mm screen to remove coarse organic matter. Duplicate, 30 g aliquots of each soil sample were adjusted to 60% field capacity and incubated in the dark at 25 ºC in a sealed mason jar that also contained an NaOH trap (3 ml, 2N NaOH) to capture the respired CO_2. The NaOH traps were titrated at regular intervals with 0.5N HCl, in the presence of $BaCl_2$ and a phenolphthalein indicator, to determine the amount of CO_2 mineralized from the soil (Horwath and Paul 1994). The NaOH traps were titrated at two-day intervals for the first two weeks of the incubation, and the interval length increased arithmetically to one-month intervals over the course of 360-day incubation period. Results from duplicate incubations were averaged to create a single data set for each replicate of each of the 9 vegetation/fire treatment combinations examined in this study.

The amount of SOC remaining in the soil after each titration was calculated by subtracting the amount of carbon lost via mineralization from the amount of total SOC

held in the soil at the previous time point. The initial concentration of SOC in each soil sample was determined as described below.

Acid Hydrolysis of the Resistant Fraction

An additional subsample of each soil composite was used to quantify the resistant SOC pool. Soil samples were passed through a 1 mm sieve, and light fraction organic matter was removed via flotation in a saturated NaCl solution (density ≈ 1.2 g cm^{-3}). Soil samples were rinsed with distilled water, dried, and picked free of any remaining recognizable plant material (Leavitt et al. 1996).

One-gram aliquots of soil were refluxed with 6N HCl at 100 °C for 18 hours. Following digestion, samples were vacuum-filtered through glass-fiber filters (Whatman GF/A, Whatman International, Maidstone, UK). The filtrate was neutralized and discarded, and the remaining soil residue was rinsed from the filter with distilled water and collected into a 50 ml centrifuge tube. Samples were rinsed with distilled water an additional three times before drying at 50 ºC.

Elemental Analysis

Organic carbon concentrations of whole soils (total SOC) and acid hydrolyzed residues (resistant SOC) were determined using a Carlo Erba NA-1500 CHN elemental analyzer (Carlo Erba Strumentazione, Milan, Italy). Whole soils were dried for 48 h at 60 °C and passed through a 2-mm sieve to remove larger organic materials. After sieving, soils were pulverized in a centrifugal mill (Angstrom, Inc., Bellville, MI). Acid hydrolyzed residues were dried and crushed with a mortar and pestle. Samples were weighed and analyzed directly with the CHN analyzer for %C. These surface soils had a neutral to nearly neutral pH (data not shown) and tested negative for the presence of $CaCO_3$; thus, output from their direct analysis yielded % SOC.

Curve Fitting to Determine SOC Pool Sizes and Turnover Rates

A two-pool exponential decay function with one constant term was used to model SOC pool sizes and turnover rates (Collins et al. 2000):

$$C_t = C_r + C_a e^{-k_a t} + C_s e^{-k_s t} \qquad \text{(Equation 1)}$$

where C_t represents the amount of SOC remaining at time t,

C_r represents the resistant C pool,

C_a and k_a represent the active C pool and its decay rate, and

C_s and k_s represent the slow-turnover C pool and its decay rate.

The model was constrained based upon direct measurements of C_t and C_r, as described above. Due to the exceptionally long mean residence times of C_r (500 to 1000 years), the exponential term for this pool is assumed to equal 1.0 and is not explicitly represented in Equation 1.

Curve fitting based upon the amount of SOC remaining over time, as determined via long-term incubation, was performed using a nonlinear regression in Sigma Plot (SigmaPlot 2002), and estimates of the active and intermediate-turnover SOC pool sizes and turnover rates were obtained. Mean residence times (MRT) were calculated as the inverse of their respective fractional rate constants ($1/k$).

Statistical Analysis

Values for each soil characteristic were averaged to the plot level (n = 3) and were log-transformed to meet the normality and equality of variance assumptions required for analysis of variance (ANOVA). Fire treatment and vegetation type (i.e. mesquite, C_3 grasses, and C_4 grasses) were considered fixed effects in a two-way ANOVA, and post hoc analysis of means was conducted using the Bonferroni procedure (SPSS 2004). P-values less than 0.05 were considered to be significantly different.

Results are presented as mean ± standard error and have been back-transformed to ease their interpretation.

Results

Soil Bulk Density

Soil bulk density differed significantly with respect to vegetation type, but not fire treatment (Table 6). Soils occurring under mesquite had significantly lower bulk density values than soils occurring under grasses ($p < 0.05$). Soils that occurred under mesquite had an average bulk density of 0.96 ± 0.02 g cm^{-3}, while those that occurred under C_3 and C_4 grasses had average bulk densities of 1.05 ± 0.01 g cm^{-3} and 1.04 ± 0.01 g cm^{-3}, respectively.

Total SOC

Total SOC concentrations were significantly greater in soils sampled under mesquite than in those sampled under C_3 or C_4 grasses ($p < 0.05$) (Table 6). Soils occurring under mesquite contained an average total SOC concentration of 17.08 ± 1.04 g C kg^{-1}soil, in contrast to soils occurring under C_3 or C_4 grasses, which contained average total SOC concentrations of 11.70 ± 0.46 and 11.36 ± 0.36 g C kg^{-1}soil, respectively. The effects of fire treatment on total SOC concentration were marginally significant ($p = 0.076$), with the soils that had been exposed to the winter and summer fire treatments having lower average total SOC concentrations than those from the unburned control (Table 7). No significant interactions between fire treatment and vegetation type were detected.

Soil Respiration Rates During the Long-term Incubation

Soil respiration rates in all treatment combinations declined exponentially from approximately 25 μg C g^{-1} soil d^{-1} during the initial days of the incubation to < 5 μg C g^{-1} soil d^{-1} after 100 days (Figure 7). The total amount of SOC mineralized over the course of the 360-day incubation ranged from 0.80 to 1.31 g C kg^{-1} soil but did not differ

TABLE 6. ANOVA results (p-values) examining the effects of fire treatment (F), vegetation type (V), and their interaction (F x V) on SOC pool sizes, MRT, and soil bulk density in the upper 10 cm of the soil profile.

Source of variation	**Total SOC** ($g\ C\ kg^{-1}$)	**Active SOC** ($g\ C\ kg^{-1}$)	**Slow SOC** ($g\ C\ kg^{-1}$)	**Resistant SOC** ($g\ C\ kg^{-1}$)	**Active MRT** (days)	**Slow MRT** (years)	**Bulk Density** ($g\ cm^{-3}$)
Fire	0.076	0.416	0.006	0.030	0.097	0.107	0.173
Vegetation	< 0.001	0.928	0.001	0.007	0.272	0.815	0.001
F x V	0.115	0.683	0.268	0.618	0.223	0.519	0.921

TABLE 7. SOC pool sizes ($\bar{x}$ ± SE, n = 3) in soils from each vegetation/fire treatment combination.

Fire	**Vegetation type**	**SOC concentration (g C kg $^{-1}$)**			
		Total	Active	Slow-turnover	Resistant
Control	Mesquite	19.43 ± 2.02	0.32 ± 0.07	13.54 ± 1.91	5.56 ± 0.06
	C_3 grasses	11.30 ± 1.13	0.27 ± 0.02	7.00 ± 0.92	4.02 ± 0.20
	C_4 grasses	11.94 ± 0.96	0.29 ± 0.04	7.52 ± 0.68	4.12 ± 0.24
Winter	Mesquite	17.91 ± 0.77	0.25 ± 0.05	9.87 ± 1.51	6.60 ± 0.93
	C_3 grasses	12.29 ± 0.25	0.30 ± 0.06	5.74 ± 1.04	6.23 ± 1.14
	C_4 grasses	11.04 ± 0.53	0.26 ± 0.03	6.00 ± 0.32	4.77 ± 0.31
Summer	Mesquite	13.91 ± 0.54	0.21 ± 0.02	6.71 ± 0.81	6.26 ± 0.55
	C_3 grasses	11.51 ± 0.97	0.25 ± 0.02	5.44 ± 0.62	5.81 ± 0.76
	C_4 grasses	11.09 ± 0.30	0.27 ± 0.03	6.28 ± 0.21	4.52 ± 0.12

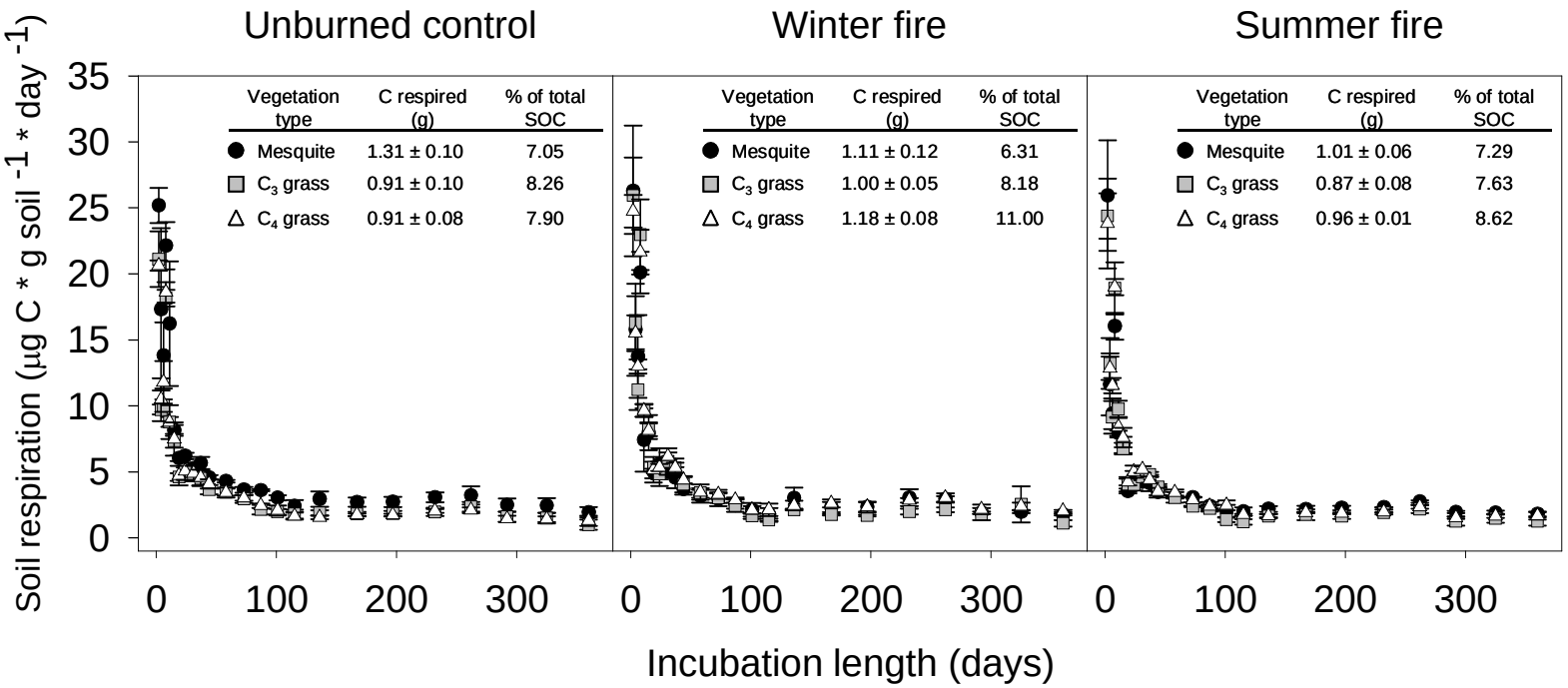

FIGURE 7. Rates of carbon mineralization ($\bar{x}$ ± SE, n = 3) during 360 days of laboratory incubation at 25 °C. Tables within each panel show the absolute amount, as well as proportion, of SOC respired during the incubation period.

significantly with respect to fire treatment or vegetation type. The total amount of carbon mineralized over the course of the incubation represented 6 to 11% of total SOC.

The Active SOC Pool

Active SOC pool size did not differ significantly with respect to fire treatment or vegetation type (Table 6). Active SOC pool sizes ranged from 0.21 to 0.32 g C kg^{-1} soil (Table 7), representing 1 to 3% of total SOC.

The Slow-turnover SOC Pool

Slow-turnover SOC pool sizes ranged from 6.00 to 13.54 g C kg^{-1} soil, representing 50 to 60% of total SOC across all vegetation types and fire treatments. As observed with total SOC, significant differences in the size of slow-turnover SOC pools were found with respect to vegetation type (Table 6). Mesquite-dominated soils contained significantly more slow-turnover SOC than did grassland soils ($p < 0.05$); however, no significant differences in slow-turnover SOC pool size were found between C_3 and C_4 grass cover types. Slow-turnover SOC stocks under mesquite averaged 10.04 ± 1.23 g C kg^{-1}soil, while those occurring under C_3 and C_4 grasses averaged 6.06 ± 0.50 g C kg^{-1} soil and 6.60 ± 0.32 g C kg^{-1} soil, respectively.

Significant differences in slow-turnover SOC pool size were also detected with respect to fire treatment (Table 6). Soils sampled from the unburned control contained significantly more slow-turnover SOC than did those from both the winter and summer fire treatments ($p < 0.05$). Slow-turnover SOC stocks from the unburned control averaged 9.35 (± 1.23) C kg^{-1} soil, while those from the winter and summer prescribed fire treatments averaged 7.02 ± 0.86 g C kg^{-1} soil and 6.14 ± 0.35 g C kg^{-1} soil, respectively.

The Resistant SOC Pool

Resistant SOC accounted for approximately 40% of total SOC and differed significantly with respect to both vegetation type and fire treatment (Table 6). Soils

occurring under mesquite tended to contain more resistant SOC than did those occurring under grasses (Table 7), and this difference was statistically significant in the case of mesquite versus the C_4 grasses. Soils that had been exposed to fire treatment, regardless of season, also tended to contain more resistant SOC than those that were unburned (Table 7). Soils that had been exposed to winter fire treatment contained significantly more resistant SOC than did those from the unburned control ($p < 0.05$). Summer fire treatment also increased the size of the resistant SOC pools relative to the unburned control, however, the statistical significance of this increase was only marginal ($p = 0.061$).

Active and Slow-turnover SOC Mean Residence Times

No significant differences were found with respect to the effects of fire treatment or vegetation type on the MRT of either active SOC or slow-turnover SOC in this system. The MRT of active SOC in this system ranged from 15 to 26 days, with an average of 19 days, and the slow-turnover MRT ranged from 6 to 12 years, with an average of 9 years (Table 8).

Discussion

The results of this study suggest that land use and land cover change both have the potential to play important roles in shaping the SOC storage and dynamics of this mixed grass savanna ecosystem. Vegetation change (i.e. the addition of mesquite to a grassland community) appears to exert direct influence over total SOC concentrations in this system, while the effects of fire treatment appear to be more indirect in nature. Soils occurring under mesquite contained significantly more total SOC than corresponding soils sampled from beneath the C_3 or C_4 grasses. In contrast, fire treatment did not alter total SOC concentrations significantly but did appear to underlie shifts in the relative contributions of the slow-turnover and resistant pools to total SOC. Slow-turnover SOC concentrations decreased by 16 to 50 % following winter or summer fire treatment,

Table 8. Rate constants (*k*) and MRT ($\bar{x}$ ± SE, n = 3) of the active and slow-turnover SOC pools from three vegetation types subject to seasonal fire treatment in a temperate mixed grass savanna ecosystem. Note that rate constants are presented on a per-day basis for active SOC and on an annual basis for slow-turnover SOC.

Fire	**Vegetation type**	**Active SOC**		**Slow-turnover SOC**	
		k-value (d^{-1})	MRT (d)	*k*-value (y^{-1})	MRT (y)
Control	Mesquite	0.064 ± 0.008	16	0.102 ± 0.017	10
	C_3 grasses	0.042 ± 0.003	24	0.094 ± 0.025	11
	C_4 grasses	0.038 ± 0.004	26	0.082 ± 0.011	12
Winter	Mesquite	0.065 ± 0.014	15	0.097 ± 0.009	10
	C_3 grasses	0.058 ± 0.010	17	0.134 ± 0.027	7
	C_4 grasses	0.065 ± 0.011	15	0.156 ± 0.025	6
Summer	Mesquite	0.051 ± 0.005	20	0.108 ± 0.029	9
	C_3 grasses	0.055 ± 0.009	18	0.120 ± 0.012	8
	C_4 grasses	0.050 ± 0.008	20	0.111 ± 0.009	9

while resistant SOC concentrations increased by 10 to 55% relative to concentrations found in the unburned control.

Vegetation Effects

Numerous studies have demonstrated the potential for woody plant encroachment to lead to the increased storage of SOC in grassland systems (Schlesinger et al. 1990, San Jose et al. 1998, Tilman et al. 2000, Hibbard et al. 2001, Pacala et al. 2001, McCulley et al. 2004, Liao et al. 2006a, Zavaleta and Kettley 2006). In order to understand the implications of this vegetation change on biogeochemical cycles and ecosystem functioning more fully, it is important to know not only how the absolute magnitude of SOC storage changes, but also what the functional characteristics of that SOC are. To this end, the results of this study suggest that the accumulation of SOC under mesquite is driven by increases in the concentration of both slow-turnover and resistant forms of SOC.

Slow-turnover SOC pool sizes occurring under mesquite were 52 to 66% larger than those sampled beneath C_3 and C_4 grasses. Similar results from ecosystems with developing or intact woody canopies suggest that new carbon tends to accumulate in the form of slow-turnover SOC. Grandy and Robertson (2007) found that surface soils (0 to 5 cm) in mid- and late-successional ecosystems in Michigan (i.e. grassland, shrubland, and forest), contained more slow-turnover SOC than soils from the annual and perennial cropping systems, as well as early successional old fields. Likewise, Liao et al. (2006a) found that a large portion of the carbon that had accumulated under mesquite along a grassland to woodland conversion chronosequence in southern Texas was in the form of slow-turnover SOC. Liao et al. (2006a) documented significant increases in the concentration of free (i.e. unprotected) particulate organic matter along their grassland to woodland chronosequence and found that this pool had an average field-based MRT of 30 years.

Although the MRT of SOC fractions associated with mesquite were slightly longer (MRT = 9.6 y) than those associated with C_3 and C_4 grasses (MRT = 8.6 and 9.0

y, respectively), no significant differences were found among slow-turnover SOC decay rates in this study. This lack of differences suggests that the increased concentrations of SOC observed under mesquite are likely to have occurred as a result of increased carbon inputs to the soil system, rather than differences in the decomposability of grass- versus mesquite-derived tissues. Lending credence to this hypothesis, it has been shown that mesquite woodlands tend to support more above- and belowground biomass and are frequently more productive than the grassland vegetation they replace (Archer et al. 2001, Hughes et al. 2006).

Resistant SOC pools quantified under mesquite were 15 to 37% larger than those found under grasses. Many SOC models assume that resistant SOC is a passive soil fraction which responds slowly to changes in vegetation and land management regime (Paul et al. 2006). However, recent evidence suggests that this pool, or some portion of it, may be more dynamic and susceptible to change than previously has been thought (McLauchlan et al. 2006, Paul et al. 2006, Cheng et al. 2007, Grandy and Robertson 2007).

Resistant SOC is typically fractionated from whole soil through the process of acid hydrolysis. Acid hydrolysis removes fatty acids, proteins, and polysaccharides from the soil matrix, leaving behind more chemically recalcitrant residues, such as long-chain alkyls, waxes, resins, lignin, and aromatic compounds (Rovira and Vallejo 2002, Paul et al. 2006). While radiocarbon analysis often dates acid hydrolyzed soil residues to ages of 1200 years or more (Falloon and Smith 2000, Paul et al. 2006), it is recognized that carbon age and chemical complexity are not necessarily synonymous (Trumbore et al. 1995). Thus, changes in the size of the resistant SOC pool may represent relatively recent inputs of complex, recalcitrant biomacromolecules, such as lignin, suberin, and other long-chain alkyls which are commonly associated with woody plants and plant roots (Nierop and Verstraten 2004, Lorenz et al. 2007), to the soil system.

Changes in belowground biomass associated with the woody plant encroachment of grassland systems have been recognized as playing an important role in altering

ecosystem carbon dynamics (Hibbard et al. 2003). Although mesquite is typically characterized as being a deep-rooted phreatophyte, it has been shown that this species is capable of developing and maintaining an extensive network of shallow lateral roots (Heitschmidt et al. 1988, Ansley et al. 1990). Mesquite-dominated sites often support 2 to 5 times more root biomass in surface soils than do remnant grassland patches (Boutton et al. 1998a, Hibbard et al. 2001). Recent work has shown that root tissue and plant litter from mesquite woodlands tend to be enriched in the syringyl and vanillyl forms of lignin, as well as the aliphatic biopolymers cutin and suberin (Boutton et al. 2008). These biochemical compounds are characterized by a high degree of resistance to biological degradation and would be characterized as resistant SOC by the acid hydrolysis method, providing a plausible explanation for the expansion of resistant SOC pools with mesquite encroachment.

Fire Effects

While vegetation type appears to be the major driver of the carbon dynamics in this system, it should be recognized that disturbances which have the ability to modify the composition and productivity of the plant community may ultimately impact ecosystem carbon dynamics as well. In the context of this study, repeated summer or winter fire treatments were not able to alter total SOC concentrations, relative to an unburned control. Although total SOC concentrations did not change significantly in response to fire treatment, the relative proportions of slow-turnover and resistant SOC did (Table 6). Slow-turnover SOC occurring under mesquite in fire treatment plots decreased by 27 to 50% relative to unburned controls, and decreases of 16 to 22% were observed under C_3 and C_4 grasses that had been exposed to fire. In contrast, resistant SOC pool sizes increased by 13 to 18% under mesquite and 10 to 55% under C_3 and C_4 grasses following fire treatment.

Increased rates of aboveground net primary productivity and biomass accumulation are often observed in temperate grassland systems following fire treatment (Knapp and Seastedt 1986, Blair 1997, Ansley et al. 2002). A fundamental assumption

of many soil organic carbon models is that such increases in productivity will trickle down to SOC pools and result in increased carbon accumulation in the soil (Fontaine et al. 2004). Although prescribed fire may stimulate net primary production, its ability to do so is not without cost to the flow of carbon to the soil system. The primary mechanism through which fire is thought to stimulate productivity is through the reduction and/or removal of preexisting biomass and litter layers (Wright and Bailey 1982, Knapp and Seastedt 1986). As the storage of organic carbon in the soil occurs as a result of the net balance between inputs to and decomposition-related losses from the soil system (Schlesinger 1997, Amundson 2001), it should be expected that disturbances which disrupt and reduce carbon inputs, even if only temporarily, may result in some degree of SOC loss.

Tiedemann and Klemmedson (1986) demonstrated such SOC loss in a mesquite removal study, in which they found that total SOC concentrations in surface soils decreased by >46%, relative to intact canopies, six years following the successful removal of mesquite trees. In cases where the mesquite trees had resprouted, total SOC concentrations were still 15% lower than those that were found under the undisturbed trees, suggesting that some minimum level of carbon input is necessary to maintain SOC stores. Evidence from cropping systems suggests similar results. Examining a range of biomass removal rates in the context of a biofuel study, Blanco-Canqui and Lal (2007) found that removing 25% or more of corn stover biomass resulted in detectable, and often significant, SOC loss.

Despite increases in both grassland (Chapter II) and mesquite (Ansley et al. 2002, Simmons et al. 2007) productivity rates in response to fire treatment at this study site, the results presented here show that the size of the slow-turnover SOC pools decreased significantly following prescribed fire treatment. While this was true for both grasses and mesquite, the largest effects seen in the slow-turnover SOC pools were associated with soils occurring under mesquite, and we attribute these losses to the disruption of inputs from aboveground biomass and litter during post-fire recovery.

In contrast to the loss of slow-turnover SOC associated with prescribed fire, resistant SOC pool sizes were observed to increase following fire treatment. Given that pyrogenic carbon (i.e. black carbon, char, or charcoal) is generally regarded as inert or highly resistant to decay, its accumulation as a result of fire treatment would seem to be a likely cause for the increases in resistant SOC observed here (Table 7). Previous studies on the effects of prescribed fire at this site have shown, however, that changes in the size of the pyrogenic carbon pool following 2 to 3 fires are not statistically significant (Dai et al. 2005, Ansley et al. 2006a). Although some charcoal does accrue in this system following fire treatment, it tends to be located deeper in the profile and is of insufficient magnitude to account for the changes in the resistant pool documented here (Dai et al. 2005, Ansley et al. 2006a).

Alternatively, the accumulation of recalcitrant biomacromolecules as a result of increased root productivity may explain the increase in size observed among the resistant carbon pools. Both Hubbard (2003) and Simmons et al. (2007) have demonstrated increased rates of root recruitment and productivity following seasonal fire treatment under both grasses and mesquite. Given the extensive root networks and belowground biomass known to exist in grassland systems and under mesquite (Sims et al. 1978, Heitschmidt et al. 1988, Hibbard et al. 2001), their ability to respond rapidly and positively to fire treatment (Ansley et al. 2002, Simmons et al. 2007), and the potential for lignin, aliphatics, and other root associated compounds to escape acid hydrolysis and be detected as a part of the resistant SOC pool, it seems reasonable that resistant SOC pools would increase under the conditions examined in this study. Thus, while fire may not contribute to the growth and maintenance of resistant SOC pools directly (i.e. through the production of pyrogenic carbon), it appears that its ability to influence plant root growth and productivity may provide an important, indirect, means for the maintenance and development of resistant SOC pools.

Conclusions

Land use and land cover change play an important role in shaping ecosystem patterns and processes. In an era in which the mitigation of atmospheric CO_2 concentrations has become increasingly important, the development, evaluation, and implementation of land use strategies and management techniques capable of enhancing soil carbon sequestration is of considerable interest. The results of this study demonstrate that both vegetation change and prescribed fire treatment play important roles in shaping the SOC dynamics of this southern Great Plains mixed grass ecosystem. Soils occurring under mesquite contained significantly more total SOC, as well as greater concentrations of slow-turnover and resistant SOC, than did soils sampled from beneath either of the C_3 or C_4 grass types. In contrast, the effects of fire did not result in changes to total SOC concentrations but did lead to altered distribution of carbon among SOC pools, resulting in increased resistant SOC concentrations but decreased slow-turnover SOC concentrations.

The enhanced accumulation of SOC under mesquite and the reallocation of carbon among SOC pools following fire treatment demonstrate the potential for carbon storage in this system, however, the long-term stability of these SOC changes is uncertain. Results from this study and others suggest that the slow-turnover SOC pool may represent a short-term carbon sink, requiring a steady flow of inputs for its perpetuation (Tiedemann and Klemmedson 1986) and maintaining strong sensitivities to disturbance (Grandy and Robertson 2007). Conversely, resistant SOC is thought to be a more stable carbon sink, and it has been suggested that directed management and species selection may be used to enhance this SOC pool for long-term carbon storage (Lorenz et al. 2007). Research is needed to characterize the chemical nature and stability of these SOC changes further, as well as determine the long-term implications they hold for ecosystem structure and function.

CHAPTER IV

SOIL MICROBIAL COMMUNITY COMPOSITION IN A SOUTHERN GREAT PLAINS MIXED GRASS SAVANNA: INFLUENCE OF PLANT FUNCTIONAL TYPES

Introduction

Land use and land cover changes frequently alter key aspects of ecosystem structure and function, affecting above- and belowground dynamics and contributing to processes of global change (Schlesinger 1997, Houghton and Goodale 2004, Jarnagin 2004, Chapin et al. 2005, Foley et al. 2005). Among the most common land use and land cover changes affecting grassland systems today is that of woody plant encroachment. Having been documented in North and South America, as well as Australia, Africa, and Southeast Asia (Van Auken 2000, Archer et al. 2001, Jackson et al. 2002, Liao et al. 2006b), woody plant encroachment of grassland systems is a geographically extensive phenomenon that has the potential to alter global nutrient cycles dramatically. Grassland systems cover approximately 40% of terrestrial land area and account for 30% of soil organic carbon on a global basis (Scurlock and Hall 1998), thus changes to their structure and function are likely to have impacts at the global scale.

In the southern Great Plains (USA), the encroachment of grassland systems by *Prosopis glandulosa* Torr. (honey mesquite), a nitrogen fixing tree, is widespread and has contributed to altered nutrient cycling and hydrologic regimes, as well as declining abundances of native grassland species (Archer et al. 2001). This change in physiognomy typically leads to increased levels of above- and belowground plant biomass, as well as increased stores of carbon and nitrogen in plants and soils (Hibbard et al. 2001, Asner et al. 2003, Dai et al. 2006, Liao et al. 2006b). Mesquite establishes extensive root networks (Sims et al. 1978, Heitschmidt et al. 1988, Hibbard et al. 2001), and its presence in grassland ecosystems often leads to the establishment of nutrient-enriched zones beneath its canopy (Tiedeman and Klemmedson 1973, Dai et al. 2006). Mesquite has the potential to access deep water stores (Scott et al. 2006) and redistribute

soil moisture to surface soil layers (i.e. conduct hydraulic lift) (Zou et al. 2005). In addition, its canopy may moderate solar radiation and soil temperatures, particularly during the peak of the summer (Tiedemann and Klemmedson 1977).

It is recognized that the aboveground and belowground components of ecosystems are intimately linked and have the potential to feedback upon one another (Wardle et al. 2004, Ehrenfeld et al. 2005, Kardol et al. 2007). The ability of individual plant species and/or assemblages to support unique microbial communities has been demonstrated in a number of systems (Bardgett et al. 1999, Smalla et al. 2001, Anderson et al. 2003, Callaway et al. 2004, Hawkes et al. 2005, Wallenstein et al. 2007) and is often attributed to differences inherent among rates of productivity and nutrient use efficiency, as well as the quantity and quality of different species' organic inputs to the soil. Mesquite-affected ecosystems often demonstrate altered levels of soil microbial biomass and activity (Reyes-Reyes et al. 2002, McCulley et al. 2004, Schade and Hobbie 2005), however, the nature and implications of these changes remains largely uncharacterized and unknown.

The objective of this study was to evaluate the structure and diversity of soil bacterial and fungal communities in a savanna-like, mesquite-encroached mixed grass prairie ecosystem. We used a direct cloning and sequencing approach and employed both distance-based and character-based phylogenetic tools to evaluate community composition, diversity, and divergence. We hypothesized that changes in ecosystem structure and function brought about by mesquite encroachment would be reflected in the composition of the soil microbial community. More specifically, we hypothesized that the composition of the bacterial and fungal communities occurring in the soil under mesquite trees would harbor a microbial community that was significantly different from those occurring under the native grasses present at this study site.

Materials and Methods

Study Site

This study was conducted in a southern Great Plains mixed grass savanna system, near Vernon, TX (33°51'20" N, 99°26'50" W). Mean annual precipitation at this site is 665 mm, bimodally distributed with peaks in May and September. Mean annual temperature is 16.1^{o} C, and monthly average extremes range from 36^{o} C in mid-summer to -2.5^{o} C in mid-winter (Ansley et al. 1990).

The soils at this site are classified as fine, mixed, superactive, thermic Vertic Paleustolls of the Tillman series, with 0 to 1% slope (Soil Survey Staff 2007). The clay loam surface soils (0 to 10 cm) have a particle size distribution of 32% sand, 52% silt, and 16% clay, and their pH values range from 7.0 to 7.2 (Ansley et al. 2006a). Estimates of aboveground net primary productivity (ANPP) (Hughes et al. 2006, Chapter II) and measured values of root biomass, soil organic carbon (SOC) content and total soil nitrogen (total N) content are given in Table 9.

The vegetative community is comprised of four major plant functional groups, each differing growth in form, phenology, productivity (Coupland 1979, Ansley et al. 2004), and tissue chemistry (Levang-Brilz and Biondini 2003). The herbaceous layer is composed of a mixture of C_3 perennial grasses, C_4 midgrasses, and C_4 shortgrasses, as well as occasional forbs and C_3 annual grasses, and the over story is dominated by mesquite. *Nassella leucotricha* (Trin. & Rupr.) Pohl (Texas wintergrass) is the dominant C_3 perennial grass species found at this site, and the dominant C_4 grasses include the midgrasses *Panicum obtusum* Kunth (vine mesquite) and *Sporobolus compositus* (Poir.) Merr. (meadow dropseed), as well as *Buchlöe dactyloides* Nutt. (buffalograss), a stoloniferous shortgrass species.

Soil Sampling

Soil cores were collected in August 2005, at which time each of the four plant types was physiologically active. Surface litter was gently brushed away and cores of mineral soil (0-10 cm, 2.5 cm diameter) were collected beneath each of the four major

TABLE 9. Site characteristics, including annual ANPP, and root biomass, as well as SOC and total N content in surface soils (0 to 10 cm), ($\bar{x}$ ± SE, n = 12). Significant differences are indicated by differing letters within each column.

Vegetation type	**ANPP** [*] ($g\ m^{-2}\ y^{-1}$)	**Root biomass** (g dry weight m^{-2})	**SOC** (g C m^{-2})	**Total N** (g N m^{-2})	**C/N ratio**
Mesquite	83 to 415	1953 ± 238 [a]	1415 ± 64 [a]	140 ± 6 [a]	10.12 ± 0.16
C_3 perennial grass	61 to 132	1843 ± 201 [ab]	1305 ± 89 [ab]	129 ± 8 [ab]	10.11 ± 0.14
C_4 midgrass	32 to 78	1579 ± 209 [b]	1148 ± 52 [b]	115 ± 5 [b]	10.08 ± 0.15
C_4 shortgrass	19 to 67	1440 ± 102 [b]	1074 ± 34 [b]	107 ± 3 [b]	10.02 ± 0.14

[*] ANPP estimates are based upon Hughes et al. 2006 for mesquite and Chapter II of this dissertation, for the grasses. Differences in ANPP rates were not assessed statistically

vegetation types represented at the study site: C_3 perennial grasses, C_4 midgrasses, C_4 shortgrasses, and mesquite. Soil cores were collected under the crowns of each grass type and at the base of each mesquite tree. Soil cores were collected in a stratified, random manner across three replicate 1 to 6 ha ungrazed pastures, and a total of 6 soil cores were collected per vegetation type. Soil samples were stored on ice in the field and were transferred to a -20 °C freezer upon return to the laboratory. Soil cores were weighed, and pairs of soil cores were combined to create three composite samples per vegetation type prior to extracting community DNA.

An additional 12 soil cores per vegetation type were collected in order to quantify root biomass. These cores were collected in the same manner as described above, and were stored at 4 °C until analysis.

Soil Organic Carbon, Soil Total Nitrogen, Bulk Density, and Root Biomass

Subsamples of each of the composite soil samples used for DNA extraction were saved for carbon and nitrogen analysis and the quantification of soil bulk density. The soil organic carbon (SOC) and total soil nitrogen concentrations of each soil composite were determined using a Carlo Erba NA-1500 CHN elemental analyzer (Carlo Erba Strumentazione, Milan, Italy). One subsample of each soil composite was dried for 48 h at 60 °C, passed through a 2-mm sieve to remove larger organic materials, and pulverized in a centrifugal mill (Angstrom, Inc., Bellville, MI). Soils were weighed and analyzed with the CHN analyzer for % C and % N. These surface soils have a neutral to nearly neutral pH (Ansley et al. 2006a) and tested negative for the presence of $CaCO_3$ (data not shown); thus, output from their direct analysis yielded % SOC.

The second subsample of each soil composite was used to determine soil bulk density. Soil samples were dried at 105 °C for 24 hours and weighed. Soil dry weights were combined with whole-core weight and volume information to back-calculate soil bulk density.

Root biomass was quantified from individual soil cores using a hydropneumatic elutriation system (Smucker et al. 1982) equipped with a 410 μm screen (Gillison's

Variety Fabrication, Inc., Benzonia, MI). Roots were dried at 60°C for 48 hours and weighed.

SOC, soil total nitrogen, and root biomass were analyzed using analysis of variance (ANOVA) (SPSS v. 12.0.2). Values were log-transformed where necessary to meet assumptions of heterogeneity and equality of variance, but back-transformed values are presented in the results to aid in interpretation. Post-hoc analysis was conducted using the Bonferonni procedure, and p-values less than 0.05 were considered to represent significant differences.

DNA Extraction and Purification

Community DNA was recovered from the composite soil samples using a modified freeze-thaw-grind method (Zhou et al. 1996, Gao et al. unpublished). Five-grams of soil were combined with 2 g of sterile sand in a sterile mortar. Samples were frozen with liquid nitrogen and ground, until they had thawed, three times. Samples were transferred to 50-ml centrifuge tubes, 13.5 ml soil extraction buffer (0.1 M PIPES [pH 6.4], 0.1M EDTA, 1.5 M NaCl, and 1% hexadecyltrimethylammonium bromide [CTAB]) were added to each sample, and then they were warmed to 65° C in a water bath. A 1.5 ml aliquot of 20% SDS was added, and samples were transferred to a 65° C incubator, being shaken horizontally at 200 rpm for one hour. Samples were then centrifuged for 10 min at 3600 x g at room temperature, and the supernatant was transferred to a new, clean centrifuge tube. Soil pellets were extracted two more times by adding 4.5 ml soil extraction buffer and 0.5 ml 20% SDS, vortexing the slurry, incubating at 65° C for 10 min, and centrifuging as above. One volume of chloroform:isoamyl alcohol (24:1) was added to the combined supernatants, mixed well, and centrifuged for 20 min at 3600 x g. The aqueous phase was collected into a 50-ml Oak Ridge tube, precipitated with 0.8 volumes of isopropanol, and allowed to incubate for 30 min at room temperature. Samples were then centrifuged at 12,000 rpm for 30 min at 25° C. The nucleic acid pellets were washed with 70% EtOH. Once dried, pellets were resuspended in 10 mM Tris (pH 8.0) and purified using a Wizard genomic

DNA purification kit (Promega Corporation, Madison, WI). Following purification, samples were desalted by adding 10 µl of 5 M NaCl and 500 µl of ice-cold EtOH (100%) and centrifuging for 15 minutes at top speed in a benchtop microfuge. All liquid was discarded, and the pellets were washed with 250 µl of 70% EtOH. The pellets were centrifuged for an additional 15 min, and pellets were dried using a vacuum centrifuge. Pellets were resuspended in 25-50 µl sterile water, and their DNA concentrations were quantified with a Nanodrop ND-1000 spectrophotometer (NanoDrop Technology, Rockland, DE) at 260 nm. DNA extracts were stored at -20° C until further analysis.

Bacterial 16S rRNA Amplification

The 16S rRNA gene was PCR amplified from community DNA samples using the general bacterial primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). Each 25 µl reaction contained 100 ng template DNA, PCR master mix (10% 10X PCR buffer, 250 µM each dNTP, 25mM $MgCl_2$, 25 µg bovine serum albumin, 0.1 µM each primer, 1.25 U *Taq* polymerase), and sterile water. Thermocycling was conducted in a GeneAmp PCR System 9700 (PerkinElmer Applied Biosystems, Norwalk, CT) under the following conditions: (i) initial denaturation at 95° C for 1 min; (ii) 30 cycles of denaturation at 94° C for 1 min, annealing at 55° C for 1 min, and extension at 72° C for 1 min, 30 sec; and (iii) a final extension at 72° C for 7 min. In order to minimize PCR biases, 5 replicate amplifications were performed for each sample and their products were combined for downstream applications, and the number of cycles utilized in the amplification process was kept low.

Fungal ITS/LSU rRNA Amplification

A portion of the fungal rRNA gene, containing the entire internal transcribed spacer (ITS) region, as well as portions of the small and large ribosomal subunits (SSU, LSU), was PCR amplified from community DNA samples using fungal-specific primers ITS9 (Egger 1995) and LSU 1221R (Schadt et al. 2003). Each 25 µl reaction contained

200 ng template DNA, PCR master mix (10% 10X PCR buffer, 200 μM each dNTP, 2.75 mM $MgCl_2$, 0.5 μM each primer, 25 μg bovine serum albumin, 1.875 U *Taq* polymerase), and sterile water. Thermocycling was conducted in a 2720 Thermal Cycler (Applied Biosystems, Foster City, CA) under the following conditions: (i) initial denaturation at 95° C for 2 min; (ii) 30 cycles of denaturation at 94° C for 45 sec, annealing at 60° C for 55 sec, and extension at 72° C for 1 min and 45 sec (+ 2 sec/cycle); and (iii) a final extension at 72° C for 10 min. As with the 16S amplifications, 5 replicate amplifications were performed for each fungal sample and their products were combined for downstream applications.

Cloning

The replicate PCR products from each original sample were combined and visualized with UV light in a 1.2% low melting point agarose gel. PCR products were excised from the gel and purified using a QiaQuick gel extraction kit (Qiagen, Valencia, CA). The purified PCR products were cloned using a TOPO TA PCR-2.1 cloning kit (Invitrogen, Carlsbad, CA) according to the manufacturer's directions, with the exception that the use of the salt solution was omitted. Transformed cells were plated onto a Luria-Bertani (LB) medium containing ampicillin (50 μg ml^{-1}) and X-gal and were incubated overnight at 37° C. The next day, approximately 150-200 positive colonies were selected from each sample, transferred to 96-well plates containing LB-ampicillin broth (50 μg ml^{-1} ampicillin), and then incubated overnight at 37° C, shaken at ~200 rpm. Plasmids were extracted from the cultured cells by boiling the cell cultures in 10 mM Tris (pH 8.0) at 99.9° C for 15 min. The plasmids were then amplified using vector primers M13F (5'-GTAAAACGACGGCCAG-3') and M13R (5'CAGGAAACAGCTATGAC-3') according to suggested procedures from the clone kit manufacturer (Invitrogen, Carlsbad, CA).

The amplified products were purified using a Montage Multiscreen filter plate (Millipore, Billerica, MA). The M13 products were transferred to the plate and filtered under a vacuum for ~15 min. Fifty microliters of 10mM Tris (pH 8.0) were added to

each well, and the samples were placed under a vacuum for an additional 10 min. An additional 25 µl of 10mM Tris were added and the plate was sealed and shaken for 30 min at 200 rpm to resuspend the M13 products. The resuspended, purified samples were collected and sequenced.

Sequencing

Purified M13 PCR products were processed with a Big Dye v3.1 sequencing kit (Applied Biosystems, Foster City, CA), and primers 8F (5'-AGAGTTTGATCCTGG-CTCAG-3'), 515F (5'-GTGCCAGC(AC)GCCGTAA-3'), 519R (5'-G(AT)ATTACC-GC(GT)GCTG-3'), and 1100R (5'-AGGGTTGCGCTCGTTG-3') were used to create overlapping sequence reads for the bacterial 16S libraries. All of the 16S clones and a portion of the fungal ITS/LSU clones (see below for primer information) were submitted to the Oak Ridge National Laboratory Environmental Sciences Division sequencing facility and were processed on an ABI 3730 sequencer (Applied Biosystems, Foster City, CA).

Purified M13 PCR products from the remainder of the fungal ITS/LSU clones were submitted to Arizona Research Laboratories, Division of Biotechnology, Genomic Analysis and Technology Core Facility (http://gatc.arl.arizona.edu) for sequencing. Big Dye (Applied Biosystems, Foster City, CA) chemistry was used, and primers ITS9 (5'-TGTACACACCGCCCGTCG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and LROF (5'-ACCCGCTGAACTTAAGC-3') were used to create overlapping sequence reads. Samples were processed on an ABI 3730 sequencer (Applied Biosystems, Foster City, CA).

Analysis

Sequence data were compiled and manually edited in Sequencher (GeneCodes 2006), and clone sequences were checked for chimeras using Chimera Check (Cole et al. 2007) and Bellerophon (Huber et al. 2004). Sequences that were identified as potentially chimeric by both programs were excluded from further analysis. The Classifier function

of the Ribosomal Database Project (Wang et al. 2007) was used for broad taxonomic characterization of the bacterial clone libraries, and BLAST searches (Altschul et al. 1990) of the Genbank database were used for taxonomic characterization of the fungal libraries.

Clone libraries were further characterized using a suite of community analysis tools to determine the degree of diversity present within, and the degree of similarity occurring between, the microbial communities present under each plant type. Separate analyses were carried out for the bacterial and fungal communities. Sequences were aligned using Clustal X (Thompson et al. 1997) and trimmed to a common length. Six hundred twenty-three aligned characters corresponding to positions 218 to 826 of the 16S rRNA of *Escherichia coli* (Cilia et al. 1996) were used in the analysis of the bacterial communities, and 445 aligned characters of the LSU corresponding to positions 67 to 440 in *Saccharomyces cerevisiae* (Bayev et al. 1981) were used as the basis for the fungal community analysis. Positions 128 to 138 and 228 to 248 of the fungal sequences were excluded from further analysis because they could not be unambiguously aligned (Schadt et al. 2003).

Diversity and Richness

Distance matrices were created in PHYLIP (Felsenstein 2004) using the DNA-DIST function and the Jukes-Cantor evolution model. Operational taxonomic units (OTUs) were defined at the 97% similarity level (McCaig et al. 1999, O'Brien et al. 2005, Schloss and Handelsman 2005), and diversity indices and richness estimates, based upon the presence and abundance of OTUs in each of the clone libraries were created using the DOTUR software package (Schloss and Handelsman 2005). Simpson's index (1/D) was used to establish a relative ranking of diversity levels among clone libraries, and Chao I was used to provide estimates of species richness that might be expected if more exhaustive sampling were to occur (Magurran 2004).

Analysis of Community Similarity and Structure

Both distance-based and character-based phylogenetic approaches were used to characterize community similarity and evaluate potential differences in community structure. Indices of community similarity, based upon shared membership and community structure were created using the SONS software package (Schloss and Handelsman 2006a). Pairwise comparisons of shared community richness, based upon OTUs, were performed for each of the vegetation-based clone libraries. Similarity in community structure was evaluated using the θ_{YC} statistic (Yue and Clayton 2005). θ_{YC} is a nonparametric maximum-likelihood estimator of similarity that accounts for the presence of shared OTUs between, as well as their relative abundances within, each community. θ_{YC} operates on a scale of 0 to 1, where 0 indicates dissimilarity and 1 represents identity.

The parsimony test (Martin 2002), as implemented by TreeClimber (Schloss and Handelsman 2006b), was used to assess whether differences in the structure of the vegetation-based microbial communities, as inferred by phylogenetics, could be attributed to random variation (i.e. by chance) or other factors (e.g. disturbance). Sequence data was pooled across clone libraries to create community-wide phylogenetic trees for the (a) bacterial and (b) fungal samples, and trees were constructed using the DNA-ML (maximum likelihood) function in PHYLIP (Felsenstein 2004). TreeClimber was used to generate both a parsimony score and a random probability distribution for each of the phylogenetic trees. P-values of less than 0.05 were considered to be statistically significant. Analogous to an ANOVA, pairwise comparisons of individual clone libraries were performed only if significant differences were detected at the whole community level first.

Nucleotide Sequence Accession Numbers

The bacterial 16S sequences used in this analysis were submitted to Genbank under accession numbers EU051919 through EU052211, and the fungal ITS/LSU sequences were submitted under accession numbers EU489881 through EU490184.

Results

Soils sampled under mesquite contained significantly more root biomass, SOC, and total N than did those sampled under the C_4 mid- or shortgrasses ($p < 0.05$) (Table 9), while soils occurring under the C_3 perennial grasses contained root biomass, SOC, and total N at levels that were intermediate in value to both the mesquite and C_4 grass samples. SOC and total N content were tightly coupled with one another in these soils; no significant differences were found with respect to soil C/N ratios.

A total of 295 bacterial sequences were produced, creating clone libraries with memberships of 54 to 83 individuals (Table 10). The bacterial libraries ranged from 47 to 71 in their abundance of unique OTUs. Related diversity statistics and richness estimators largely reflected these differences in library size (Table 10), with the bacterial community characterized under mesquite reflecting greater diversity and estimated richness levels than the C_3 perennial grass, C_4 midgrass, or C_4 shortgrass communities. Phylum-level characterization of the 16S clones showed that the libraries were largely composed of individuals representing the *Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Acidobacteria*. These four phyla accounted for 95% of library membership. The remaining 5% included members of the *Gemmatimonadetes*, *Bacteriodetes*, *Cyanobacteria*, and *Nitrospira* (Figure 8).

In the characterization of the soil fungal community, 304 sequences were produced, resulting in clone libraries that ranged in size from 72 to 78 individuals (Table 10). Each of the fungal libraries contained 20 to 33 unique OTUs, with the fungal communities occurring under mesquite and C_3 perennial grass appearing to harbor greater levels of diversity and estimated richness than those occurring beneath the C_4 mid- and shortgrasses. At the phylum-level, the fungal libraries were dominated by members of the *Ascomycota*, composed to a lesser degree by members of the *Zygomycota* and *Basidiomycota*, and contained few *Chytridiomycota* (Figure 9).

TABLE 10. Clone library size, number of OTUs identified, Simpson's index, and Chao I estimated richness values for the (A) bacterial and (B) fungal clone libraries sampled from the soil beneath 4 major mixed grass savanna vegetation types.

Plant functional type	Library size	OTUs identified*	Simpson (1/D)	Chao I estimated richness
(A) Bacteria				
Mesquite	83	71	226.87	341
C_3 perennial grass	54	47	178.87	184
C_4 midgrass	75	54	75.00	162
C_4 shortgrass	83	68	170.15	245
(B) Fungi				
Mesquite	77	33	13.06	63
C_3 perennial grass	77	28	10.68	79
C_4 midgrass	78	20	3.02	50
C_4 shortgrass	72	22	8.30	33

*OTUs were defined as sequences sharing ≥ 97% similarity and served as the basis for the Simpson's and Chao I calculations.

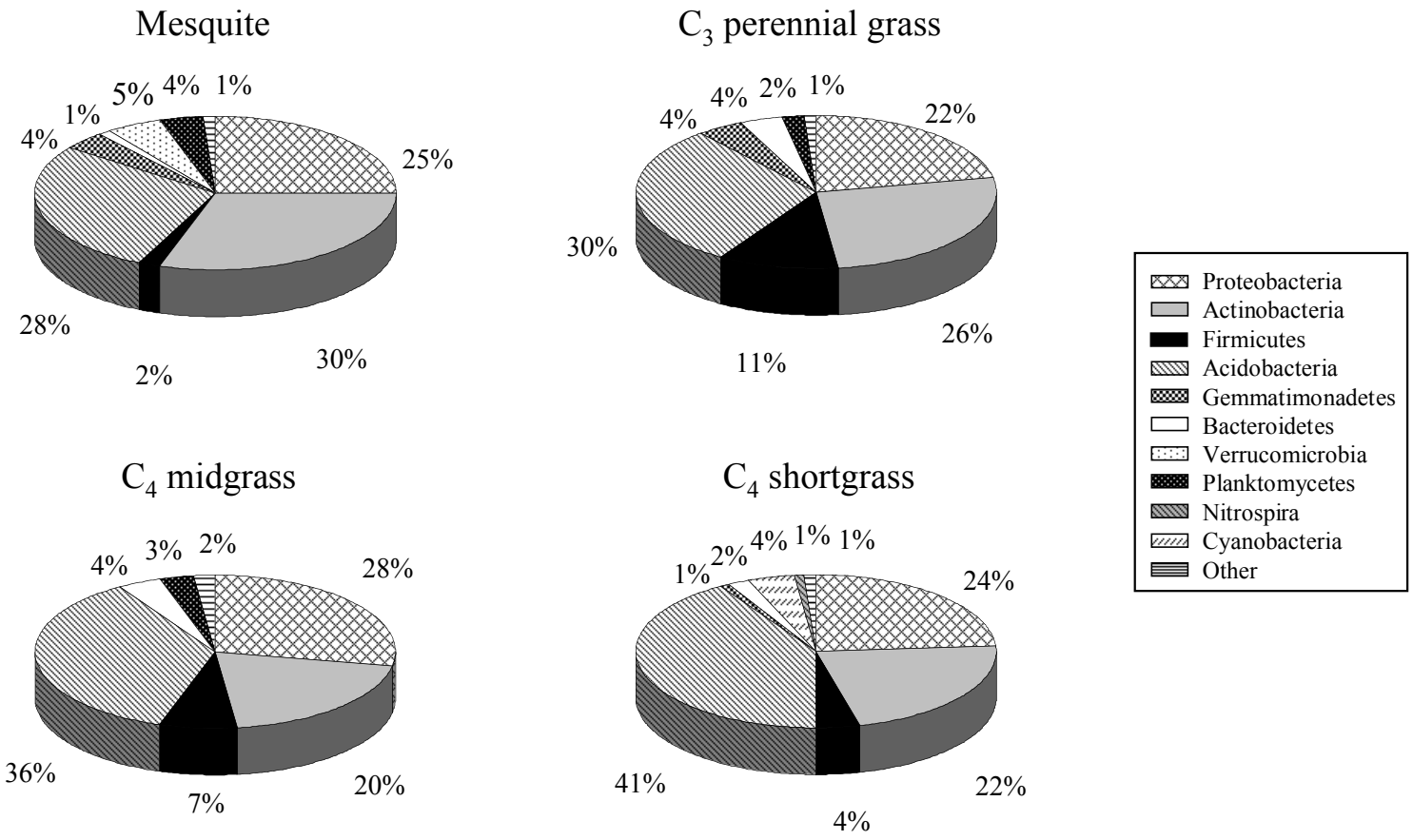

FIGURE 8. Distribution and relative abundance of bacterial phyla among the four vegetation based clone libraries.

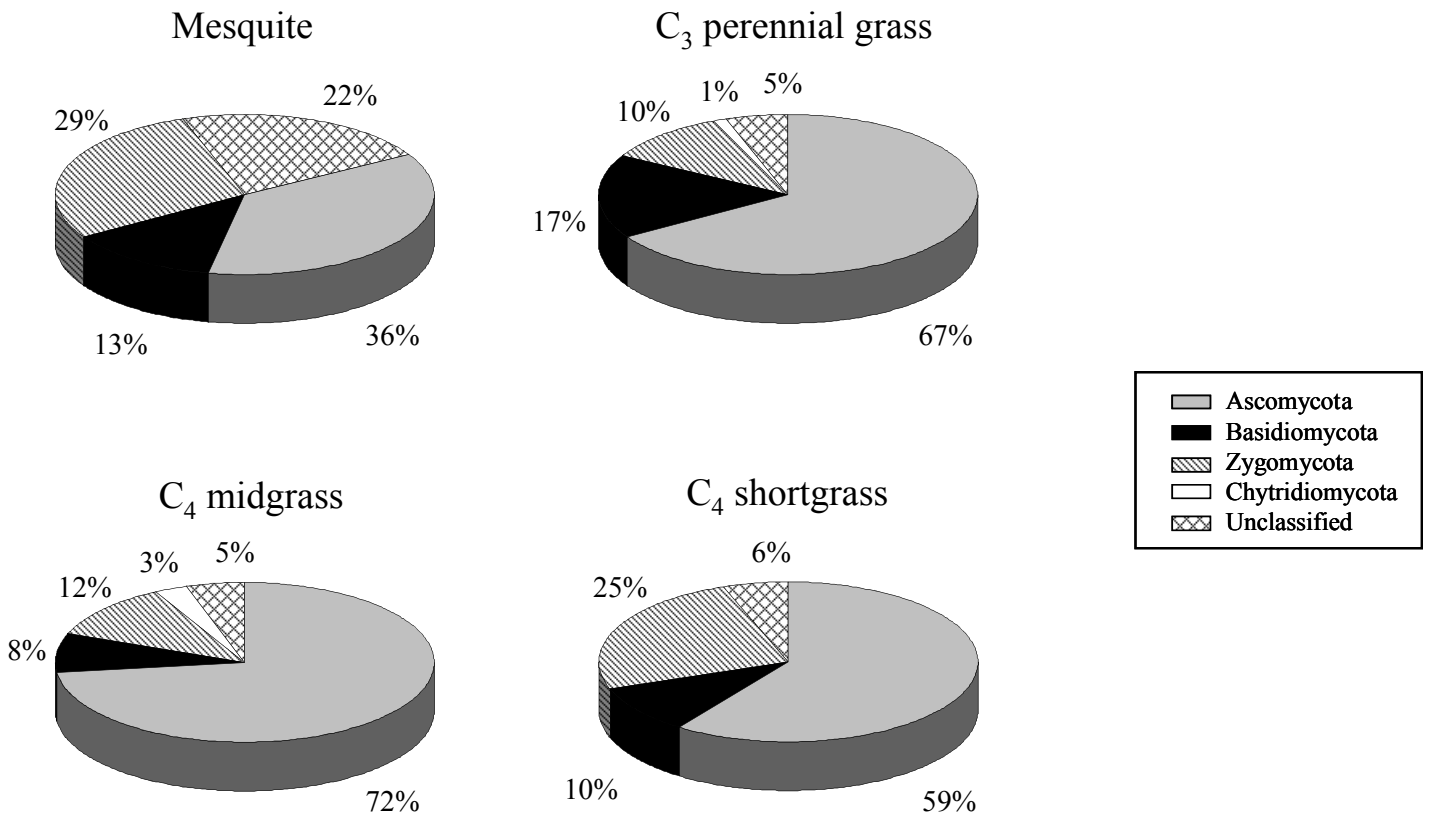

FIGURE 9. Distribution and relative abundance of fungal phyla among the four vegetation based clone libraries.

Analysis of Community Similarity and Structure

Pairwise comparisons of shared community richness performed using the SONS software program found that 6 to 32% of bacterial and 18 to 54% of fungal OTUs were shared among the four vegetation-based clone libraries (Table 11). Overall similarity in community structure for each of the paired clone libraries, as represented by θ_{YC}, ranged from 0.034 to 0.276 among the bacterial clone libraries and 0.399 to 0.620 among the fungal clone libraries (Table 11).

The analysis of community differences using the phylogeny-based parsimony test detected no significant differences in phylogenetic structure of the bacterial community (Table 12). In contrast, the parsimony test did identify significant differences in phylogeny within the fungal community (Table 12). Pairwise comparisons of each of the fungal clone libraries found that the mesquite soil fungal community harbored a phylogenetic structure that was significantly different from those associated with the soil fungal communities associated with C_3 perennial grasses ($p = 0.037$), C_4 midgrasses ($p = 0.033$), and C_4 shortgrasses ($p = 0.041$). None of the fungal communities associated with each of the grass types was significantly different from one another.

Discussion

At broad levels of taxonomic characterization, the composition of the clone libraries associated with each of our four prairie vegetation types was quite similar. Members of the phyla *Proteobacteria, Actinobacteria, and Firmicutes* were found in all 16S clone libraries (Figure 8) and represented the affiliations of more than 80% of the bacterial clones sequenced in this study. These results closely match the findings of a recent synthesis of soil-based bacterial clone library composition, in which members of the phyla *Proteobacteria* and *Actinobacteria* were commonly identified and found to represent an average of 39% (range, 10 to 77%) and 13% (range, 0 to 34%) of library membership, respectively (Janssen 2006). The same review found that members of the phylum *Firmicutes*, including representatives of the genera *Bacillus* and *Clostridium*, typically represent an average of 2% (range, 0 to 8%) of library composition across all

TABLE 11. Shared operational taxonomic units (OTUs) and the Yue-Clayton pairwise community similarity index (θ_{YC}) for the soil (A) bacterial and (B) fungal communities as calculated by SONS at a genetic distance of 0.03.

Vegetation pair (X,Y)	Proportion of shared OTUs *		Community similarity
	X_Y	Y_X	θ_{YC}) ± SE **
(A) *Bacteria*			
Mesquite – C_3 perennial grass	0.056	0.083	0.034 ± 0.02
Mesquite – C_4 midgrass	0.098	0.125	0.134 ± 0.05
Mesquite – C_4 shortgrass	0.155	0.162	0.097 ± 0.04
C_3 perennial – C_4 midgrass	0.125	0.107	0.062 ± 0.03
C_3 perennial – C_4 shortgrass	0.250	0.176	0.142 ± 0.05
C_4 midgrass – C_4 shortgrass	0.321	0.265	0.276 ± 0.07
(B) *Fungi*			
Mesquite – C_3 perennial grass	0.294	0.312	0.399 ± 0.10
Mesquite – C_4 midgrass	0.177	0.250	0.403 ± 0.11
Mesquite – C_4 shortgrass	0.265	0.346	0.575 ± 0.11
C_3 perennial – C_4 midgrass	0.312	0.417	0.507 ± 0.12
C_3 perennial – C_4 shortgrass	0.438	0.538	0.462 ± 0.10
C_4 midgrass – C_4 shortgrass	0.417	0.385	0.620 ± 0.12

* X_Y, the proportion of OTUs in library X shared with library Y. Y_X, the proportion of OTUs in library Y shared with library X.
** θ_{YC} is scored on a scale of 0 to 1, with a score of 0 being dissimilar and a score of 1 being identical.

TABLE 12. Parsimony test scores and their significance for the (A) bacterial and (B) fungal communities as computed by TreeClimber. Significance values indicate the likelihood of differences in community structure among the clone libraries being due to chance (null hypothesis).

Vegetation pair	Parsimony score	Significance
(A) *Bacteria*		
All bacterial samples	146	0.078
(B) *Fungi*		
All fungal samples	135	0.045
Mesquite – C_3 perennial grass	37	0.037
Mesquite – C_4 midgrass	36	0.033
Mesquite – C_4 shortgrass	35	0.041
C_3 perennial – C_4 midgrass	44	0.063
C_3 perennial – C_4 shortgrass	41	0.056
C_4 midgrass – C_4 shortgrass	43	0.086

soil types but are often more abundant in clone libraries constructed from grassland soils (range, 17 to 52%) (Janssen 2006). The relative abundance of *Firmicutes* ranged from 15 to 34% among our four vegetation-based bacterial clone libraries (Figure 8).

Like the bacterial clone libraries, the fungal clone library composition also shared a high degree of similarity at broad taxonomic levels. Members of the *Ascomycota* represented 42 to 76% of soil fungal clones, while members of the *Zygomycota* (9 to 31%), *Basidiomycota* (9 to 24%), and *Chytridiomycota* (0 to 2%) were less commonly encountered (Figure 9). These values are similar to those reported in a recent study of fungal diversity in tallgrass prairie, in which Jumpponen and Johnson (2005) found that *Ascomycota* represented 53 to 71% of fungal clones in bulk and rhizosphere soils, while *Basidiomycota* (24 to 40%), *Zygomycota* (3 to 7%), and *Chytridiomycota* (0 to 1%) occurred less frequently.

Despite the similarities shared among the bacterial and fungal clone libraries at broad taxonomic levels, further characterization suggests that their resemblance with one another may be somewhat limited at finer scales. In addition, such resemblance may be dependent upon the perspective from which it is considered. Estimates of community similarity (θ_{YC}), based upon the presence of shared OTUs and their relative abundances, ranged from 0.034 to 0.276 among the bacterial clone libraries and from 0.399 to 0.620 among the fungal clone libraries (Table 11). These results demonstrate that core groups of bacterial and fungal OTUs occur commonly throughout the soils of our study site; however, they also reveal that a substantial degree of dissimilarity exists with respect to the composition and structure of each of the vegetation-based clone libraries at finer scales.

It might be expected that communities sharing a low degree of similarity in their OTU composition (i.e. membership) would also differ with respect to their phylogenetic diversity. While such a scenario has the potential to occur, OTU composition and phylogenetic diversity are not inherently linked, as each considers genetic information in a different manner. In describing the parsimony test, Martin (2002) provides an example in which the reader is asked to consider, "…two communities that do not have any

sequences in common; however, for every closely related sequence in the [first] community, there is a closely related sequence in the other community." Under these circumstances, the degree of OTU overlap between the two communities would be minimal, their phylogenetic diversity would be virtually identical, and the interpretation of their similarities or differences would depend upon the method(s) one chose to use.

The bacterial communities in this study appear to be quite dissimilar with respect to their OTU composition; however, the parsimony test failed to find statistically significant differences in their phylogenetic structure (Table 12). The combination of these results may be interpreted as: (a) an indication that a high level of redundancy exists within the soil bacterial communities at this site, or (b) the result of limited statistical power. Although we were able to characterize a diverse range of soil bacteria, our efforts were certainly not exhaustive. The estimated richness of these communities may be in excess of 340 OTUs, yet our richest library contained just slightly more than $1/5^{th}$ of that number of OTUs (Table 10). This, combined with the marginally significant result of the bacterial community parsimony test ($p = 0.078$), suggests that additional sampling and characterization would be likely to reveal distinct and significant differences in the structure of the bacterial community.

In contrast with the bacteria, the parsimony test identified significant differences among the fungal communities (Table 12). Further analysis showed that the community occurring under mesquite was significantly different from each of the other fungal communities characterized in this study. These results are further demonstrated by the distribution and relative abundance of taxa among the soil fungal communities. The community occurring under mesquite supported a greater proportion of *Zygomycota* and unclassified fungi, but a lesser proportion of *Ascomycota*, than did the clone libraries associated with each of the other vegetation types (Figure 9).

Although some members of the *Zygomycota* are better known as "sugar molds" because they lack the enzyme capacity necessary to break down complex organic compounds, the *Zygomycota* actually represent a broad range of saprobes commonly associated with plant litter and soils (Alexopoulos et al. 1996). A recent study of plant

associated soil microbial communities in a woody encroachment scenario in the arctic documented increased abundance of *Zygomycota* in shrub soils relative to remnant tussock vegetation (Wallenstein et al. 2007). As woody plants establish themselves in grassland systems, the changes they impart are likely to require unique, and potentially more diverse, microbial assemblages to adequately process organic inputs. The results of this study and Wallenstein et al. (2007) suggest that zygomycetes may be important components of such assemblages.

Mesquite woodlands not only support greater rates of productivity and biomass accumulation than the grassland vegetation they replace (Archer et al. 2001, Hughes et al. 2006), they also maintain substantial differences in their tissue chemistry. Relative to grassland species, the biomass produced in mesquite woodlands tends to be enriched in biochemically recalcitrant macromolecules, such as the syringyl and vanillyl forms of lignin and the aliphatic biopolymers cutin and suberin (Boutton et al. 2008). Microcosm studies have shown that increased fungal diversity may lead to the more efficient degradation of recalcitrant substances (Setälä and McLean 2004), and studies of soil fungal communities from other aggrading shrub- and woodland systems have demonstrated distinct shifts in community structure with changing vegetation type (Anderson et al. 2003, Wallenstein et al. 2007). Although the implications for such changes are largely still unknown, they serve to demonstrate the complexity of natural ecosystems, as well as provide fuel for speculation regarding their potential to influence biogeochemical cycles and other ecosystem processes.

Increasing evidence suggests that that the linkages shared between plant species and microbial communities may be important contributing factors to the success of plant species establishment, invasion, and persistence (Klironomos 2002, Mitchell and Power 2003, Callaway et al. 2004, Hawkes et al. 2005, Hawkes et al. 2006). The continued characterization of plant-microbe associations may aid in our understanding of plant community development, ecological succession, and exotic species invasion and could lead to new techniques for ecosystem management, restoration, and invasive species control.

CHAPTER V

EVALUATION OF THE FUNCTIONAL GENE DIVERSITY OF SOIL COMMUNITIES IN A SOUTHERN GREAT PLAINS MIXED GRASS SAVANNA USING THE GEOCHIP, A FUNCTIONAL GENE MICROARRAY

Introduction

Land use and land cover changes frequently alter key aspects of ecosystem structure and function, affecting above- and belowground dynamics and contributing to processes of global change (Schlesinger 1997, Houghton and Goodale 2004, Jarnagin 2004, Chapin et al. 2005, Foley et al. 2005). Among the most common land use and land cover changes affecting grassland systems today is that of woody plant encroachment. Having been documented in North and South America, as well as Australia, Africa, and Southeast Asia (Van Auken 2000, Archer et al. 2001, Jackson et al. 2002, Liao et al. 2006b), woody plant encroachment of grassland systems is a geographically extensive phenomenon that has the potential to alter global nutrient cycles dramatically. Grassland systems cover approximately 40% of terrestrial land area and account for 30% of soil organic carbon on a global basis (Scurlock and Hall 1998), thus changes to their structure and function are likely to have impacts at the global scale.

In the southern Great Plains (USA), the encroachment of grassland systems by *Prosopis glandulosa* Torr. (honey mesquite), a nitrogen fixing tree, is widespread and has contributed to altered nutrient cycling and hydrologic regimes, reductions in ecosystem stability, and declining abundance of native grassland species (Archer et al. 2001). This change in physiognomy typically leads to enhanced levels of above- and belowground plant biomass (Hibbard et al. 2001, Asner et al. 2003), altered rates of net primary productivity (Hughes et al. 2006, Hollister et al., unpublished data), and increased stores of carbon and nitrogen in plant tissue and soils (Dai et al. 2006, Liao et al. 2006b). Mesquite establishes extensive root networks (Sims et al. 1978, Heitschmidt et al. 1988, Hibbard et al. 2001), and its presence in grassland ecosystems often leads to the establishment of nutrient-enriched zones beneath its canopy (Tiedeman and

Klemmedson 1973, Dai et al. 2006), as well as the accumulation of biochemically recalcitrant compounds, such as the syringyl and vanillyl forms of lignin and the aliphatic biopolymers cutin and suberin (Boutton et al. 2008). Mesquite-encroached ecosystems often demonstrate altered levels of soil microbial biomass and activity (McCulley et al. 2004, Schade and Hobbie 2005, Scott et al. 2006), however, the nature of these changes within the soil microbial community and implications that they hold for ecosystem function remain largely uncharacterized and unknown.

Changes in plant cover type, fertilization regime, and climate have been shown to alter the composition of soil microbial communities (Nusslein and Tiedje 1999, Smalla et al. 2001, Schadt et al. 2003, Grayston et al. 2004, Bohme et al. 2005, Artz et al. 2007, Wallenstein et al. 2007). It is thought that such changes might also be accompanied by shifts in community functional capacity, but to date, testing such hypotheses has proven to be difficult. Approaches that have been used include metabolic profiling (e.g. Zak et al. 1994b) and enzyme assays (e.g. Nannipieri et al. 2002), quantitative and reverse-transcription PCR (e.g. Nogales et al. 2002, Burgmann et al. 2003), and the construction and analysis of clone libraries (e.g. Rondon et al. 2000). Although these methods have provided valuable insight into the functioning of soil microbial communities, their ability to characterize community diversity fully tends to be limited by cultivation-related biases and/or the labor- and cost-intensive nature of gene-by-gene approaches (Gentry et al. 2006, Loy et al. 2006).

In recent years, microarray technology has emerged as a powerful tool for the detection and analysis of gene expression patterns. Microarrays provide a specific, sensitive, high throughput medium through which the expression patterns of thousands of genes can be analyzed simultaneously, and they offer a substantial degree of flexibility in their design (Gentry et al. 2006, Loy et al. 2006). These aspects readily lend the application of microarray technology to the study of complex systems, however, it has only been in the last few years that the use of microarrays has been extended to the study of natural environments (Loy et al. 2002, Rhee et al. 2004, Franke-Whittle et al. 2005, Brodie et al. 2006, DeSantis et al. 2007, Yergeau et al. 2007).

The two major types of microarrays that have been developed for use in natural environments include those designed to track community composition using phylogenetic markers such as the 16S RNA gene (Loy et al. 2002, Brodie et al. 2006, DeSantis et al. 2007) and those designed to monitor community function. This second type of array, also known a functional gene array (FGA), contains probes for genes that are thought to be involved in biogeochemical cycling and other environmental processes (Wu et al. 2001). FGAs provide a means for evaluating the genetic capacity and functional diversity harbored within complex communities (Zhou and Thompson 2002, Gentry et al. 2006). The most comprehensive FGA created to date is known as the GeoChip (He et al. 2007). It is constructed of 50-mer oligonucleotide probes and contains over 24,000 gene probes, representing more than 10,000 genes and 150 functional groups known to be involved to carbon, nitrogen, phosphorus and sulfur cycling, as well as the degradation of organic contaminants and metal reduction/resistance (He et al. 2007).

The objective of this study was to characterize the functional diversity of soil microbial communities in a savanna-like, mesquite-encroached temperate mixed grass prairie ecosystem. We used the GeoChip to characterize the functional diversity of soil microbes occurring under mesquite and three grass functional types. We hypothesized that soil microbial communities occurring under mesquite would have functional gene abundance profiles that differed from those of the microbial communities occurring under grasses. More specifically, we expected the greatest differences in functional gene abundance would occur among genes related to nitrogen cycling and the metabolism of carbon compounds.

Materials and Methods

Study Site

This study was conducted in a southern Great Plains mixed grass savanna system, near Vernon, TX (33°51’20” N, 99°26’50” W). Mean annual precipitation at this site is 665 mm, bimodally distributed with peaks in May and September. The mean annual

temperature is 16.1^{o} C, and monthly average extremes range from 36^{o} C in mid-summer to -2.5^{o} C in mid-winter (Ansley et al. 1990). The soils at this site are classified as fine, mixed, superactive, thermic Vertic Paleustolls of the Tillman series, with 0 to 1% slope (Soil Survey Staff 2007).

The vegetative community is comprised of four major plant functional groups, each differing growth in form, phenology, productivity (Coupland 1979, Ansley et al. 2004), and tissue chemistry (Levang-Brilz and Biondini 2003). The herbaceous layer is composed of a mixture of C_3 perennial grasses, C_4 midgrasses, and C_4 shortgrasses, as well as occasional forbs, and the over story is dominated by mesquite. *Nassella leucotricha* (Trin. & Rupr.) Pohl (Texas wintergrass) is the dominant C_3 perennial grass species found at this site, and the dominant C_4 grasses include the midgrasses *Panicum obtusum* Kunth (vine mesquite) and *Sporobolus compositus* (Poir.) Merr. (meadow dropseed), as well as *Buchlöe dactyloides* Nutt. (buffalograss), a stoloniferous shortgrass species.

Soil Sampling

Soil cores were collected in August 2005, at which time each of the four plant types was physiologically active. Surface litter was gently brushed away and cores of mineral soil (0-10 cm, 2.5 cm diameter) were collected beneath each of the four major vegetation types represented at the study site: C_3 perennial grasses, C_4 midgrasses, C_4 shortgrasses, and mesquite. Soil cores were collected under the crowns of each grass type and at the base of each mesquite tree. Soil cores were collected in a stratified, random manner across three replicate 1 to 6 ha ungrazed pastures, and a total of 6 soil cores were collected per vegetation type. Soil samples were stored on ice in the field and were transferred to a -20 °C freezer upon return to the laboratory. Soil cores were weighed, and pairs of soil cores were combined to create three composite samples per vegetation type prior to extracting community DNA.

An additional 12 soil cores per vegetation type were collected in order to quantify root biomass. These cores were collected in the same manner as described above, and were stored at 4 °C until analysis.

Soil Carbon and Nitrogen, Bulk Density, and Root Biomass

Subsamples of each of the composite soil samples used for DNA extraction were saved for carbon and nitrogen analysis and the quantification of soil bulk density. The soil organic carbon (SOC) and total soil nitrogen (total N) concentrations of each soil composite were determined using a Carlo Erba NA-1500 CHN elemental analyzer (Carlo Erba Strumentazione, Milan, Italy). One subsample of each soil composite was dried for 48 h at 60 °C, passed through a 2-mm sieve to remove larger organic materials, and pulverized in a centrifugal mill (Angstrom, Inc., Bellville, MI). Soils were weighed and analyzed with the CHN analyzer for %C and %N. These surface soils have a neutral to nearly neutral pH (Ansley et al. 2006a) and tested negative for the presence of $CaCO_3$ (data not shown); thus, output from their direct analysis yielded % SOC.

The second subsample of each soil composite was used to determine soil bulk density. Soil samples were dried at 105 °C for 24 hours and weighed. Soil dry weights were combined with whole-core weight and volume information to back-calculate soil bulk density.

Root biomass was quantified from individual soil cores using a hydropneumatic elutriation system (Smucker et al. 1982) equipped with a 410 μm screen (Gillison's Variety Fabrication, Inc., Benzonia, MI). Roots were dried at 60°C for 48 hours and weighed.

DNA Extraction and Purification

Community DNA was recovered from the composite soil samples using a modified freeze-thaw-grind method (Zhou et al. 1996, Gao et al. unpublished). Five-grams of soil were combined with 2 g of sterile sand in a sterile mortar. Samples were frozen with liquid nitrogen and ground until they had thawed three times. Samples were

transferred to 50-ml centrifuge tubes, 13.5 ml soil extraction buffer (0.1 M PIPES [pH 6.4], 0.1M EDTA, 1.5 M NaCl, and 1% hexadecyltrimethylammonium bromide [CTAB]) were added to each sample, and they warmed to 65° C in a water bath. A 1.5 ml aliquot of 20% sodium dodecylsulfate (SDS) was added, and samples were transferred to a 65° C incubator, being shaken horizontally at 200 rpm for one hour. Samples were then centrifuged for 10 min at 3600 x g at room temperature, and the supernatant was transferred to a new, clean centrifuge tube. Soil pellets were extracted two more times by adding 4.5 ml soil extraction buffer and 0.5 ml 20% SDS, vortexing the slurry, incubating at 65° C for 10 min, and centrifuging as above. One volume of chloroform:isoamyl alcohol (24:1) was added to the combined supernatants, mixed well, and centrifuged for 20 min at 3600 x g. The aqueous phase was collected into a 50-ml Oak Ridge tube, precipitated with 0.8 volumes of isopropanol, and allowed to incubate for 30 min at room temperature. Samples were then centrifuged at 12,000 rpm for 30 min at 25° C. The nucleic acid pellets were washed with 70% EtOH. Once dried, pellets were resuspended in 10 mM Tris (pH 8.0) and purified using a Wizard® genomic DNA purification kit (Promega, Madison, WI). Following purification, samples were desalted by adding 10 µl of 5 M NaCl and 500 µl of ice-cold EtOH (100%) and centrifuging from 15 minutes at top speed in a benchtop microfuge. All liquid was discarded, and they pellets were washed with 250 µl of 70% EtOH. The pellets were centrifuged for an additional 15 min, and pellets were dried using a vacuum centrifuge. Pellets were resuspended in 25-50 µl sterile water, and their concentration was quantified with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technology, Rockland, DE) at 260 nm. DNA extracts were stored at -20° C until further analysis.

Amplification and Labeling

Whole community amplification of genomic (soil) DNA samples was performed using a TempliPhi 500 amplification kit (Amersham Biosciences, Piscataway, NJ) (Wu et al. 2006). Each 24 µl reaction contained 50 ng genomic DNA, 10 µl manufacturer's sample buffer containing random hexamers, 10 µl manufacturer's reaction buffer

containing deoxynucleotides, 1 µl enzyme mixture, 1 µl *Escherichia coli* single-strand binding protein (SSB, 267 ng/µl), and 1 µl spermidine (0.1 mM). Samples were incubated at 30 °C for 4 hr, followed by heat inactivation of the enzyme at 65 °C for 10 min. Amplification products were visualized in a 1% agarose gel under UV light.

Amplified DNA was fluorescently labeled with Cy5-dUTP using a random priming method (Rhee et al. 2004). For each reaction, target DNA was combined with 0.06 µg random primer, 0.3 µl spermidine (10 mM) and distilled, deionized water (ddH_2O) to a volume of 40 µl. Samples were denatured at 99.9 °C for 5 min and then immediately chilled on ice. Labeling master mix (20 µl), containing 5mM (each) dATP, dCTP, and dGTP; 2.5 mM dTTP; 1 mM Cy5-dUTP, 343 ng recA, 40 U Klenow fragment, and ddH_2O, was added to each sample and incubated at 37 °C for 6 hr, in the dark. Labeled DNA products were purified with a QIAquick PCR purification column (Qiagen, Valencia, CA) according to its manufacturer's protocols. Samples were dried using a vacuum centrifuge and stored at -20 °C.

Hybridization

All hybridizations were carried out in duplicate, and each replicate was prepared and processed independently. Microarray slides, coverslips, pipette tips, and hybridization chambers were warmed to 50 °C prior to hybridization. The fluorescently labeled DNA samples were mixed with a hybridization solution containing: 50% formamide; 5X SSC (1X SSC is equal to 150 mM NaCl plus 15mM trisodium citrate); 0.10% SDS; 4 µg unlabeled herring sperm DNA; 0.33 µl 1mM spermidine; and ddH_2O in a total volume of 40 µl. The hybridization mixture was incubated at 95 °C for 5 min and then kept on at heating block at 50 °C to prevent cross-hybridization. Just prior to the application of samples to the arrays, 1.6 µl of RecA (490 µg ml^{-1}) were added to the hybridization solution. Microarray slides were placed in self-contained hybridization chambers (Corning Life Science, Corning, NY), coverslips were added, and samples were pipetted onto each array and allowed to spread across them via capillary action. The hybridization chambers were sealed and incubated in a 50 °C water bath overnight.

Following incubation, each microarray slide was removed from its hybridization chamber, and its cover slip was removed by immersion in a pre-warmed (50 °C) washing buffer (1X SSC and 0.01% SDS). Arrays were incubated for 5 min at 50 °C in a fresh aliquot of this buffer. Arrays were then incubated in a second washing buffer (0.1X SSC and 0.1% SDS) for 10 min at room temperature. A third washing buffer (0.1X SSC) was used in a series of final washing steps, in which slides were incubated in four changes of buffer for 1 min at room temperature with gentle shaking. Arrays were dried using compressed N_2 followed by centrifugation at 500 x *g* in a clean, padded slide box, at room temperature (Rhee et al. 2004).

Image Processing and Data Analysis

A ScanArray Express microarray analysis system (Perkin-Elmer, Wellesley, MA) was used to scan the microarrays at a resolution of 10 μm. Scanned images were saved as 16-bit TIFF files and were processed using the ImaGene 6.0 software package (BioDiscovery, El Segundo, CA). A grid of circles corresponding to the location of each DNA probe on the microarray was overlaid onto the TIFF images and aligned with the known location of embedded controls. The mean hybridization signal intensity for each spot was quantified, and local background signals were subtracted from the signal of each spot. Poor-quality hybridization spots were automatically flagged by ImaGene, and additional spots were removed based upon their signal to noise ratio (SNR). SNR was calculated as (mean signal intensity – mean background intensity)/ standard deviation of the background (Wu et al. 2006), and spots with SNR values < 2 were excluded from further analysis (Loy et al. 2002).

Signal intensity values were normalized by mean, and outliers were removed according to the method outlined by He et al. (2007). Data from each of the technical replicates were combined with one another, and gene detection was considered successful if a positive hybridization signal for a given probe occurred across both of the technical replicates. Normalized signal intensity values were then averaged across technical replicates and used in downstream analysis.

The scope of this study is limited to the functional genes contained in the "A" portion of the array, which is summarized in Table 13. This portion of the dataset contains probes for the entire suite of functional genes that is targeted by the GeoChip, however it lacks the additional variants for each gene that are contained on the full microarray.

Analysis of variance was used to compare signal intensities and gene abundances, as well as root biomass, soil organic carbon and total soil nitrogen content, and carbon-to-nitrogen ratio relative to vegetation type (SPSS Inc, Chicago, IL). Values were log-transformed where necessary to meet assumptions of heterogeneity and equality of variance, but back-transformed values are presented in the results to aid in interpretation. Post-hoc analysis was conducted using the Bonferonni procedure, and p-values less than 0.05 were considered to represent significant differences.

Analysis of similarity (ANOSIM) was used to compare the similarity of functional gene abundance patterns across vegetation types, and these relationships were visualized in a dendrogram using hierarchical clustering analysis (HCA). ANOSIM is a nonparametric statistical test which ranks the elements of a dissimilarity matrix and compares the mean difference of ranks between groups and within them, generating the statistic *R* (Clarke 1993, Ramette 2007). Values of R range from -1 to +1, with negative values and values near 0 indicating similarity among samples and values approaching +1 indicating a strong dissimilarity among samples. Bray-Curtis dissimilarity values were calculated among samples (Legendre and Legendre 1998), replicate samples were grouped by vegetation type, and ANOSIM was conducted using the PAST software program (Hammer et al. 2001). PAST was also used to conduct the HCA, in which Pearson's correlation coefficient and the paired-group algorithm served as the basis for clustering gene abundance profiles among samples.

TABLE 13. Summary of functional gene array content and the distribution of probes successfully detected among the soil microbial communities characterized in this study.

Gene functional group	**Number of genes on the array**	**Number of gene probes**	**Probes detected in this study**
Carbon degradation	980	1014	56
Carbon fixation	376	428	14
Methane cycle	303	303	13
Nitrogen cycle	1988	2027	82
Sulfur cycle	627	646	20
Metal reduction/resistance	1610	1933	128
Organic remediation	2774	2774	178
Total (*including others not specifically listed*)	8658	9170	494

Results

Soils sampled under mesquite contained significantly more root biomass, SOC, and total N than did those sampled under the C_4 mid- or shortgrasses ($p < 0.05$) (Table 14), while soils occurring under the C_3 perennial grasses contained root biomass, SOC, and total N at levels that were intermediate in value to both the mesquite and C_4 grass samples. SOC and total N content were tightly coupled with one another in these soils; no significant differences were found with respect to soil C/N ratios.

A total of 494 FGA probes were successfully detected across all samples, representing genes and gene families from each of the functional groups that were included on the microarray (Table 13). A full listing of these genes, their sources of origin, Genbank accession numbers, and their detection among vegetation types can be found in Appendix A. Genes involved in organic contaminant degradation were detected with the greatest abundance, followed by those related to metal resistance, the nitrogen cycle, and the degradation of carbon compounds. Few significant differences were found among samples with respect to: a) the number of gene probes detected; b) their aggregate signal intensities; or c) the number of gene probes detected relative to their taxonomic origin (Table 15). The only significant differences that were detected were related to the abundance of genes involved in nitrification (Table 15), in which greater numbers of nitrification genes were detected among C_4 midgrass samples, relative to mesquite and the C_4 shortgrasses.

Further analysis of functional gene profiles via ANOSIM found no significant differences among our four soil microbial communities with respect to their gene abundance and signal intensity patterns (Table 16). This lack of differences is further illustrated by the distribution of samples among clusters in the HCA dendrogram (Figure 10), in which no individual vegetation type clustered independently from any of the others.

TABLE 14. Annual aboveground net primary productivity (ANPP) and surface soil characteristics (0 to 10 cm) related to the four vegetation type functional groups evaluated in this study.

Vegetation type	ANPP ($g\ m^{-2}\ y^{-1}$)	Root biomass (g dry weight m^{-2})	SOC (g C m^{-2})	Total N (g N m^{-2})	C/N ratio
Mesquite	83 to 415	1953 ± 238 [a]	1415 ± 64 [a]	140 ± 6 [a]	10.12 ± 0.16
C_3 perennial grasses	61 to 132	1843 ± 201 [ab]	1305 ± 89 [ab]	129 ± 8 [ab]	10.11 ± 0.14
C_4 midgrasses	32 to 78	1579 ± 209 [b]	1148 ± 52 [b]	115 ± 5 [b]	10.03 ± 0.16
C_4 shortgrasses	19 to 67	1440 ± 102 [b]	1074 ± 34 [b]	107 ± 3 [b]	10.02 ± 0.14

Significant differences are represented by differing letters within each column. ANPP estimates are based upon Hughes et al. 2006 for mesquite and Chapter II of this dissertation, for the grasses. Differences in ANPP rates were not evaluated statistically.

TABLE 15. Results of ANOVA tests evaluating the effects of aboveground vegetation type on belowground microbial community functional gene abundance.

Response Variable	**Significance (p-value)**	
	Number of probes detected	Summed signal intensity
Gene functional group		
Carbon degradation	0.175	0.168
Carbon fixation	0.143	0.551
Methane cycle	0.246	0.224
Nitrogen fixation	0.221	0.168
Nitrification	0.037	0.082
Nitrogen reduction	0.210	0.286
Sulfur cycle	0.326	0.320
Organic degradation	0.150	0.136
Metal resistance/reduction	0.112	0.150
Gene probe origin		
Archaea	0.126	0.059
Bacteria	0.133	0.133
Eukaryota	0.310	0.229
Lab clone/unclassified	0.066	0.101

TABLE 16. ANOSIM results: pairwise *R*-distances with Bonferroni-corrected p-values shown in parentheses.

Vegetation type	Mesquite	C_3 perennial grasses	C_4 short-grasses	C_4 mid-grasses
Mesquite				
C_3 perennial grasses	0.083 (1.00)			
C_4 shortgrasses	-0.250 (1.00)	-0.222 (1.00)		
C_4 midgrasses	0.417 (0.60)	0.185 (1.00)	0.185 (0.58)	

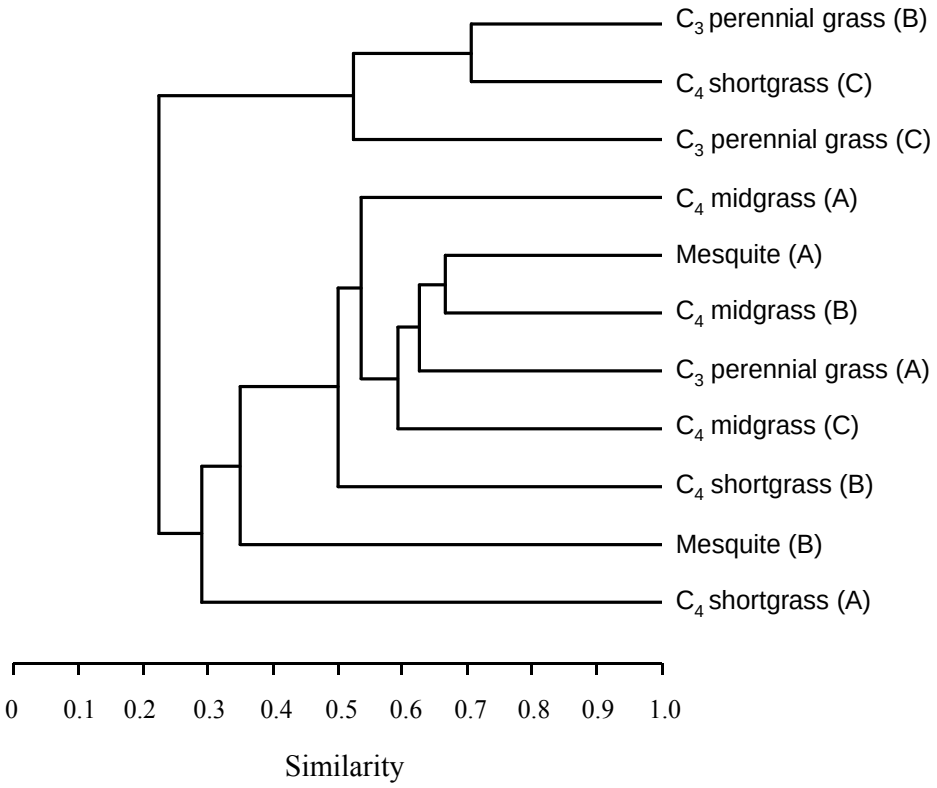

FIGURE 10. Hierarchical clustering of functional gene abundance profiles and their similarity with one another. Similarity scores approaching 1.0 represent absolute similarity, and scores approaching 0 represent absolute dissimilarity. Biological replicates within individual vegetation types are indicated by the letters in parentheses.

Discussion

Despite the differences that exist among the four plant functional groups examined in this study with respect to growth form, phenology, productivity, tissue chemistry, and the nutrient content of their associated soils, few differences were found with respect to the functional gene capacity of their associated soil microbial communities. Not only were gene probes detected in similar numbers across all samples, their signal intensity also occurred at similar levels, and they shared a common distribution across gene types. These results may suggest that the soil microbial communities examined in this study share a high degree of functional redundancy. Alternatively, these results could be interpreted as suggesting that the functional differences harbored by the communities we examined are outside of the scope of the GeoChip.

Support for the case of functional redundancy is often based on the observation that ecosystems harbor large numbers of species relative to the number of processes they carry out. It is thought that the occurrence of functional redundancy may be commonplace within soil microbial communities (O'Donnell et al. 2005), given: a) the high degree of taxonomic diversity that has been shown to exist among them (Torsvik et al. 1990); b) the ability to perform a number of basic processes being broadly distributed among taxa (Schimel 1995, Zak et al. 2006); and c) and the saturation of species number-ecosystem function curves at fairly low numbers of microbial taxa (Setälä and McLean 2004). Functional redundancy provides a form of biological insurance, ensuring that essential functions will continue to be carried out in the face of fluctuating environmental conditions (Naeem and Li 1997, Yachi and Loreau 1999), and it is thought to be an indicator of successional status and ecosystem health (Yin et al. 2000).

Although it is an unanticipated result given the ongoing process of land cover change and the differences known to exist among the vegetation types we examined, it is certainly possible that the functional capacity of the soil microbial communities in this ecosystem has been unaffected and is broadly redundant. Such an interpretation should be accepted with caution, however, as the use of microarrays is inherently limited by: a)

our current level of understanding regarding functional pathways and processes, and b) the quality and extent of the information contained within the molecular databases upon which most microarrays are based (Loy et al. 2006). Although we have learned a great deal about ecosystem structure and function through the use of the GeoChip (Rodríguez-Martínez et al. 2006, He et al. 2007, Reeve 2007, Yergeau et al. 2007) and other microarray platforms, we are very much still working with an incomplete dataset.

A recent example of this can be found in Zhang et al. (Zhang et al. 2007) with their demonstration of the use of an FGA along a forest development chronosequence. Although Zhang et al. (2007) successfully detected numerous functional genes with their array, they found few significant differences among the microbial communities they examined. Despite their FGA being among the most comprehensive arrays currently available for the study of organic carbon degradation, Zhang et al. (2007) concluded that additional research was necessary in order to improve our understanding of the genetic diversity and functional pathways associated with decomposition processes.

One of the greatest challenges associated with the design and use of microarrays for environmental samples is our continued inability to account for the vast number of unknown and uncharacterized DNA sequences that habitats such as soil, air, and water are thought to contain (Gentry et al. 2006). If an organism, or gene, of ecological importance is not represented within existing molecular databases, it is unlikely to be accounted for in microarray design, and it is further unlikely to be detected when the array is applied to a sample (i.e. system) of interest. Likewise, even if organisms, or genes, do exist within the molecular databases, but they are not included in the scope and design of a microarray, their utility is lost. With this in mind, we expect that a number of potential differences in our study system were beyond the scope of the GeoChip and thus, were not accounted for.

Studies of systems undergoing succession suggest that as an ecosystem matures, its microbial community is likely to become increasingly dominated by fungi (Bardgett et al. 2005). Parallel analysis (via cloning and sequencing) of the same microbial communities examined in this study found statistically significant differences among

vegetation types with respect to the composition of their associated soil fungal communities, but failed to find significant differences among soil bacteria (Chapter IV). While cloning and sequencing efforts, based upon ribosomal RNA, and FGA characterizations are not directly comparable to one another, their combined results are interesting to consider in light of the fact that the majority of the gene probes used in the design of the GeoChip are of bacterial (~98%), rather than fungal (~2%) origin (He et al. 2007). The development of an FGA which places greater emphasis on functional genes of fungal origin, such as manganese peroxidase (*mnp*), lignin peroxidase (*lip*), and glyoxyl oxidase (*glx*) (Zak et al. 2006), might provide greater insight into the function of this soil microbial community than did the current version of the GeoChip.

Conclusions

It is appears to be a rule, rather than an exception, that the continued characterization of microbial communities results in the discovery of new taxa (Schloss and Handelsman 2004, Jumpponen and Johnson 2005). With such discovery also comes the potential for the discovery of novel enzymes, pathways, and functional genes, as well as new perspectives on functional diversity. While the use of microarrays in environmental studies is still in its infancy, this technology has proven to be a powerful tool for the characterization and monitoring of soil microbial communities (Loy et al. 2002, Rhee et al. 2004, Tiquia et al. 2004, Reeve 2007, Yergeau et al. 2007). Although the results of this study failed to find significant differences in the functional gene capacity of soil microbial communities occurring under different prairie vegetation types, it is anticipated that the continued development of this technology and the knowledge bases which support it will result in the creation of improved, habitat-specific microarrays capable of provide greater insight into the structure and function of soil microbial communities and the connections they share with the broader environment.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Ecosystem structure and function are governed by myriad linkages and interactions. The overarching goal of this study was to identify and characterize relationships shared among the components of a mixed grass savanna ecosystem which might be relevant to controlling the flow of carbon through the plant-soil system and which might also be altered by land use and land cover change. The results of this study demonstrate the occurrence of substantial changes in community composition and function as a result of mesquite (*Prosopis glandulosa* Torr.) encroachment and seasonal prescribed fire treatment. They provide the most thorough characterization of aboveground net primary productivity (ANPP) in the southern Great Plains region to date, as well as a detailed analysis of the functional characteristics of soil organic carbon (SOC) and its response to disturbance. In addition, this study provides novel information regarding soil community responses to the widespread vegetation change phenomenon of woody plant encroachment.

Past studies have shown that the process of mesquite encroachment has the potential to affect multiple ecosystem properties and processes, including ecosystem carbon and nitrogen storage, soil microbial biomass levels and activity, and hydrologic processes (Tiedeman and Klemmedson 1973, Archer et al. 2001, Hibbard et al. 2001, McCulley et al. 2004, Zou et al. 2005, Ansley et al. 2006a, Dai et al. 2006, Liao et al. 2006b). The results of this study further demonstrate these effects and provide information which may be useful in guiding land use and management decisions. The results of Chapter II show that the ANPP of the herbaceous plant community was not significantly different between open grassland and mesquite subcanopy areas; however, the composition of those of these two areas was. Although C_3 perennial grasses occurred evenly and abundantly throughout the study plots, open grassland areas supported a greater proportion of C_4 mid- and shortgrasses relative to mesquite subcanopy areas, while the mesquite subcanopies supported increased abundances of C_3

annual grasses and forbs. While these changes in community composition may have little impact from the perspective of aboveground net primary productivity, the replacement of deep-rooted C_4 midgrass species by more shallow-rooted C_3 annual grasses and forbs has the potential to alter belowground productivity, as well as nutrient cycling dynamics (Hobbie 1992).

Other effects that have been attributed to mesquite encroachment include altered rates and distributions of ANPP (Hibbard et al. 2003, Hughes et al. 2006), as well as increased storage of SOC (Liao et al. 2006b). While the storage of SOC following woody plant encroachment of grassland systems has been a subject of dispute among authors, the results of Chapter III provide evidence documenting increased carbon storage in surface soils (0 to 10 cm) following mesquite encroachment in this mixed grass savanna ecosystem. Soils occurring under mesquite not only contained more total SOC, but they also supported greater concentrations of slow-turnover and resistant SOC, relative to soils occurring beneath either of the C_3 or C_4 grass functional types. Increases in the slow-turnover pool are likely to be a consequence of increased rates of organic matter input under mesquite, relative to the species in the open grassland areas (Hughes et al. 2006), while increases in the resistant SOC pool may be related to mesquite root biomass (Boutton et al. 1998b, Hibbard et al. 2001) and its biochemistry (Paul et al. 2006, Boutton et al. 2008).

Although mesquite encroachment has the potential to alter ecosystem properties and processes dramatically, these effects and others may be altered, attenuated, or removed altogether as a result of human interaction or other disturbances. In the case of this study, the effects of prescribed fire treatment were examined with respect to their potential to influence plant community composition, ANPP, and SOC storage. The results of Chapter II demonstrate that both winter and summer fire treatments led to increased rates of ANPP (average 434 and 313 g m^{-2} y^{-1}, respectively), relative to that of the unburned control (average, 238 g m^{-2} y^{-1}). Fire treatment also resulted in altered plant community composition. Sites that had been exposed to fire treatment supported

greater abundances of, and increased productivity rates among, C_3 forb species and C_4 mid- and shortgrasses.

Fire treatment not only altered plant community composition productivity, it also led to changes in SOC storage (Chapter III). While overall SOC concentrations did not change significantly following fire treatment, significant changes were found with respect to the distribution of SOC among its functional pools. The proportion of slow-turnover SOC declined following fire treatment, while the resistant SOC fraction was found to increase. The decline of slow-turnover SOC pools is attributed to fire's removal of aboveground biomass and litter and, thus, it's disruption of organic matter inputs from aboveground sources. In contrast, the growth of the resistant SOC fraction is thought to be the result of increased organic matter inputs belowground. More specifically, as fire treatment tends to stimulate root biomass and productivity (Hubbard 2003, Simmons et al. 2007), the accumulation of recalcitrant biomacromolecules such as lignin, suberin, and other long-chain alkyls which are commonly associated with plant roots (Nierop and Verstraten 2004, Lorenz et al. 2007) is likely to contribute to SOC storage.

Ultimately, changes in plant community composition and organic matter inputs, whether occurring through succession or encroachment, or as a result of directed land management activities, have the potential to alter soil microbial communities, their functional attributes, and the relationships they share with aboveground plant communities. The results of Chapter IV demonstrate the occurrence of diverse soil microbial communities within this mixed grass savanna ecosystem. Although numerous studies in the literature have demonstrated the ability of individual plant species to harbor unique microbial communities (Bardgett et al. 1999, Smalla et al. 2001, Anderson et al. 2003, Callaway et al. 2004, Hawkes et al. 2005, Wallenstein et al. 2007), no significant differences were found within the bacterial portion of the soil microbial communities in this ecosystem. These findings may be limited by the number of bacterial sequences that were able to be gathered during the course of this study, and further sequencing efforts may ultimately reveal significant differences among the

bacterial communities associated with each of the vegetation types present in this mixed grass savanna ecosystem. However, the lack of differences in bacterial clone library composition compliments the findings of Chapter V, in which few significant differences in functional gene capacity were found among plant-based soil microbial communities using the GeoChip functional gene microarray (FGA). Taken together, these results suggest that there may be a high degree of functional redundancy within the bacterial portion of the soil microbial community within this ecosystem.

Although the GeoChip is the most comprehensive FGA currently available, one of its potential shortcomings is that it only contains ~2% fungal genes, by origin (He et al. 2007). Thus, it is has limited ability to assess the functional capacity of soil fungi or their response to changing environmental conditions. Interestingly, the significant differences that were detected among the soil microbial communities in this study occurred among the fungi (Chapter IV).

The fungal community associated with mesquite was significantly different from those associated with each of the other vegetation types. It supported increased abundances of *Zygomycota* and unclassified fungi, but reduced abundances of *Ascomycota*, relative to the fungal communities associated with each of the other vegetation types. Studies of soil fungal communities from other aggrading shrub- and woodland systems have demonstrated similarly distinct shifts in community structure with changing vegetation type (Anderson et al. 2003, Wallenstein et al. 2007), and microcosm studies have shown that increased fungal diversity may lead to the more efficient degradation of recalcitrant carbon compounds (Setälä and McLean 2004). While the functional consequences of such change are not completely understood, it is likely that they will have important implications for the cycling of carbon and other nutrients through plants, soils, and the atmosphere. In addition, changes in fungal diversity could potentially alter plant species interactions with their biological and physical environment, thereby influencing biogeochemical cycling, successional processes, and the biodiversity of plant communities in this region (Bever et al. 1997, van der Heijden et al. 1998, Lemons et al. 2005).

Changes to community components and processes have important consequences for ecosystem structure and function. They have the potential to alter the linkages and relationships shared among ecosystem components. Furthermore, they influence the ways in which we assign value to lands and make decisions regarding their use and management; and they affect our ability to model and predict ecosystem scenarios and outcomes in the future. The results of this study demonstrate the occurrence of substantial ecosystem changes as a result of mesquite encroachment and seasonal prescribed fire treatment and provide key information to aid our understanding of the consequences of land use and land cover change.

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APPENDIX A

MICROARRAY GENE PROBES AND DETECTION AMONG SAMPLES

TABLE A-1. Target, description, and origins of functional genes detected using the GeoChip functional gene microarray. Successful detection of genes within one or more biological replicates of each sample (M – Mesquite, C_3 – C_3 perennial grass, 4S – C_4 shortgrass, 4M – C_4 midgrass) is indicated by a plus sign (+). Non-detection is indicated by a minus (-) sign.

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Carbon degradation								
Cellulose	Cellulase	*Bacillus subtilis*	13785983	13785984	+	+	+	+
	Cellulase	*Clostridium thermocellum*	40675	581006	+	+	+	+
	Cellulase	*Piromyces sp.* E2	25990954	25990955	+	+	+	+
	Cellulase	*Xanthomonas axonopodis*	21240774	21244241	-	-	+	-
	Cellulase	*Xanthomonas campestris*	21229478	21231202	-	-	-	+
	Cellulase	*Reticulitermes speratus*	8926964	8926965	+	+	+	+
	Cellulase	*Reticulitermes speratus*	8926982	8926983	+	-	-	-
	Cellulase (putative)	*Salmonella typhimurium*	16763390	16764956	+	+	+	+
	Endoglucanase	*Thermobifida fusca*	1817722	1817723	+	+	+	+
	Endoglucanase	*Leuconostoc mesenteroides*	28876819	23028615	-	-	+	+
	Endoglucanase	*Leuconostoc mesenteroides*	28876819	23028996	-	+	-	+
	Endoglucanase	*Agrobacterium tumefaciens*	17936711	17937015	-	-	-	+
	Endoglucanase	*Chaetomium brasiliense*	21842087	21842088	-	-	-	+
	Endoglucanase	*Halobacterium sp.* NRC-1	15789340	15790492	-	+	-	-
	Endoglucanase	*Thermotoga maritima*	15642775	15644497	+	+	+	+
	Endoglucanase	*Trametes hirsuta*	38175313	38175314	+	-	-	-
	Endoglucanase	*Trichoderma viride*	21842123	21842124	-	-	+	+
	Endoglucanase (probable)	*Pirellula sp.* 1	32470666	32475429	+	+	-	-
	Endoglucanase	*Bacillus sp.* KSM-330	142872	121839	-	+	-	+
	Endoglucanase	*Sulfolobus solfataricus*	15896971	15898195	+	+	+	+
Chitin	Chitinase	*Ajellomyces capsulatus*	11935133	11935134	-	-	-	+
	Chitinase	*Botryotinia fuckeliana*	22255888	22255889	+	+	+	+
	Chitinase	*Burkholderia gladioli*	7209515	7209516	+	+	+	-
	Chitinase	*Coccidioides immitis*	31295885	31295886	-	-	-	+
	Chitinase	*Plasmodium vivax*	37590963	37590964	+	+	+	-
	Chitinase	*Rhizopus niveus*	218024	218025	+	+	-	+

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Chitin	Chitinase	*Salinivibrio costicola*	33284729	33284730	-	-	-	+
	Chitinase	*Stenotrophomonas maltophilia*	2952288	2952289	+	-	-	+
	Chitinase	*Thermococcus chitonophagus*	38176263	38176264	-	-	+	+
	Chitinase	*Thermococcus kodakaraensis*	6580044	6580045	+	+	+	+
	Chitinase	uncultured bacterium	18182565	18182566	+	+	+	+
	Chitinase	uncultured bacterium	18182589	18182590	+	+	+	+
	Chitinase	*Xanthomonas sp.* AK	4115620	4115621	+	+	+	+
	Chitinase (predicted)	*Pseudomonas syringae*	28876508	23471663	+	+	+	+
	Chitinase (putative)	*Salmonella enterica*	16758993	16759224	-	-	-	+
	Chitinase	*Shewanella oneidensis*	24371600	24374814	+	+	-	+
	Chitinase	*Neurospora crassa*	9368570	9368583	-	+	-	-
	Endochitinase	*Trichoderma atroviride*	32170386	32170387	+	-	+	+
Lignin	Lignin	*Daedalea quercina*	34223693	34223694	+	+	+	+
	Lignin	*Gaeumannomyces graminis*	19171197	19171198	-	+	-	-
	Lignin	*Ganoderma sp.* BS-1	37813116	37813117	-	-	+	-
	Lignin	*Pleurotus ostreatus*	4098194	4098195	+	-	-	-
	Lignin	*Pleurotus sajor-caju*	32399648	32399649	-	+	-	-
	Lignin	*Trametes sp.* C30	37702650	37702651	-	+	+	+
	Lignin	*Trametes villosa*	1100245	1100246	+	+	+	+
	Lignin	uncultured basidiomycete	18073071	18073072	-	-	+	+
Mannan	Beta-mannanase	*Thermobifida fusca*	3970819	3970820	-	+	-	-
	Beta-mannosidase	*Thermotoga neapolitana*	14718750	14718751	-	-	+	-
	Endo-1,4-beta-mannanase	*Bacillus subtilis*	13249155	13249156	+	+	+	+
	Endo-1,4-beta-mannanase	*Cellvibrio japonicus*	28200472	28200473	-	+	-	+
	Endo-1,4-beta-mannanase	*Rhodothermus marinus*	2832599	2832600	+	+	-	+
Phospho-gluconolactone	6-phosphoglucono-lactonase (putative)	*Mycobacterium leprae*	15826865	15827228	+	-	-	-
Polysaccharides	Polygalacturonase	*Penicillium griseoroseum*	6289087	6289088	-	-	+	-
	Polygalacturonase	*Penicillium olsonii*	5441843	5441844	+	-	-	-

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Polysaccharides *(cont'd)*	Polygalacturonase	*Phytophthora cinnamomi*	22750434	22750435	+	+	+	+
Carbon fixation								
acetyl coA	Acetyl-CoA decarbonylase/synthase	*Methanosarcina thermophila*	12408703	12408704	+	-	-	-
	Acetyl-CoA synthetase	*Bacillus subtilis*	32468809	2650695	+	+	-	-
	Biotin carboxyl carrier	*Sulfolobus metallicus*	2792513	2826314	-	-	-	+
Carbon monoxide	Carbon monoxide dehydrogenase	*Methanopyrus kandleri*	19886933	38257684	+	-	-	-
	Carbon monoxide dehydrogenase	*Carboxydothermus hydrogenoformans*	10802691	10802692	-	+	-	-
	Carbon monoxide dehydrogenase	*Methanocaldococcus jannaschii*	1591436	38257392	-	-	-	+
Formate--tetrahydrofolate	Formate--tetrahydrofolate ligase	*Bacteroides thetaiotaomicron*	29345410	29346147	-	-	-	+
	Formate--tetrahydrofolate ligase	*Shewanella oneidensis*	24371600	24372154	+	-	-	-
Carbon dioxide	RuBisCO	*Rhodobacter sphaeroides*	22959343	22961807	-	-	-	+
	RuBisCO	*Archaeoglobus fulgidus*	11497621	11499228	+	+	+	+
	RuBisCO	*Rhodospirillum rubrum*	46404	132036	-	-	-	+
	RuBisCO	*Thioalkalispira microaerophila*	38479560	38479561	-	-	+	+
	RuBisCO	*Synechococcus sp.*	1850937	1850940	-	-	+	+
Succinyl-CoA	Succinyl-CoA synthetase	uncultured episymbiont of *Alvinella pompejana*	34592276	34592277	-	-	-	+
Methane cycle								
Methane	Methyl coenzyme C reductase	*Methanococcus vannielii*	150054	225629	+	+	+	+
	Methyl coenzyme M reductase	uncultured archaeon	34305111	34305113	+	+	-	+
	Methyl coenzyme M reductase	uncultured methanogenic archaeon	34017055	34017056	+	+	-	+
	Methyl coenzyme M reductase (putative)	*Methanothermus fervidus*	149796	293192	-	+	-	-

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Methane *(cont'd)*	Soluble methane monooxygenase hydroxylase	*Methylomonas sp.* KSPIII	6002396	6002398	+	+	-	+
	Soluble methane monooxygenase	uncultured bacterium	37813019	37813020	+	+	+	+
	Particulate methane monooxygenase	thermophilic methanotroph	4105846	4105847	-	+	-	+
	Particulate methane monooxygenase	uncultured bacterium	34017081	34017082	+	+	+	-
	Particulate methane monooxygenase	uncultured bacterium	22324382	22324383	+	-	-	+
	Particulate methane monooxygenase	uncultured bacterium	22324388	22324389	+	+	-	+
	Particulate methane monooxygenase	uncultured bacterium	27529178	27529179	+	+	+	+
	Particulate methane monooxygenase	uncultured eubacterium pAMC512	6424872	6424923	+	-	+	+
Nitrogen fixation								
N_2	Nitrogenase	*Alcaligenes faecalis*	1183861	1183862	+	+	+	+
	Nitrogenase	*Clostridium cellulolyticum*	1816462	1816463	-	-	-	+
	Nitrogenase	*Methanopyrus kandleri*	20093440	20094852	+	+	+	+
	Nitrogenase	*Methanosarcina barkeri*	3810902	3810903	-	-	+	+
	Nitrogenase	*Paenibacillus macerans*	2897666	2897667	+	+	+	+
	Nitrogenase	*Treponema primitia*	12659195	12659196	-	-	+	-
	Nitrogenase	uncultured bacterium	15187028	15187029	+	+	+	+
	Nitrogenase	uncultured bacterium	33385608	33385609	-	-	+	-
	Nitrogenase	uncultured microorganism SN-13	6523534	6523535	-	+	+	-
	Nitrogenase	uncultured microorganism SN-40	6523542	6523543	-	-	+	+
	Nitrogenase	uncultured nitrogen-fixing bacterium	19070146	19070147	-	-	+	-
	Nitrogenase	uncultured prokaryote	21586616	21586617	-	-	+	+

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
N_2 *(cont'd)*	Nitrogenase	uncultured prokaryote	21586696	21586697	+	+	+	+
	Nitrogenase	unidentified marine eubacterium	780712	780713	-	+	+	+
	Nitrogenase	unidentified nitrogen-fixing bacteria	3157561	3157562	+	+	+	+
	Nitrogenase	unidentified nitrogen-fixing bacteria	3157721	3157722	-	-	+	+
Ammonification/Immobilization								
Glutamate	Glutamate dehydrogenase	*Bacillus halodurans*	15612563	15616504	+	-	+	+
	Glutamate dehydrogenase	*Deinococcus radiodurans*	15805042	15806721	+	+	+	+
	Glutamate dehydrogenase	*Gloeobacter violaceus*	37519569	37520702	-	+	-	+
	Glutamate dehydrogenase	*mitochondrion Trypanosoma brucei*	4106518	4106519	+	+	+	+
	Glutamate dehydrogenase	*Porphyra yezoensis*	32480564	32480565	+	+	+	+
Urea	Hydrogenase/urease accessory protein	*Desulfitobacterium hafniense*	30470694	23118827	+	+	-	+
	Hydrogenase/urease accessory protein	*Leuconostoc mesenteroides*	28876819	23042919	+	+	+	-
	Hydrogenase/urease	*Rhodobacter sphaeroides*	22959343	22981255	-	-	+	-
	Urea amidohydrolase	*Xylella fastidiosa*	22997051	23000617	-	-	+	-
	Urea amidohydrolase	*Magnetospirillum magnetotacticum*	23015057	23020535	-	-	+	-
	Urea amidohydrolase	*Leuconostoc mesenteroides*	28876819	23027996	+	+	-	+
	Urea amidohydrolase	*Pseudomonas syringae*	28876509	23471416	+	+	-	+
	Urea amidohydrolase	*Nostoc punctiforme*	30581879	23129838	-	+	-	-
	Urea amidohydrolase	*Rhodobacter sphaeroides*	22958664	22958708	+	+	+	+
	Urease	*Caulobacter crescentus*	13423248	13423257	-	+	-	+
	Urease	*Vibrio parahaemolyticus*	6063402	6063403	+	+	+	+
	Urease	*Bacillus sp.*	393296	216360	+	+	+	+
	Urease	*Deinococcus radiodurans*	6460670	6460755	+	+	+	+
	Urease	*Helicobacter salomonis*	21637209	21637211	-	-	+	+
	Urease	*Nostoc sp.* PCC 7120	17132540	38605406	-	-	+	-
	Urease	*Photorhabdus luminescens*	36785405	36785518	+	+	+	-

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Urea *(cont'd)*	Urease	*Synechococcus sp.* WH 8102	33639324	33639578	-	-	-	+
	Urease	*Thermosynechococcus elongatus*	22297544	22297873	+	+	+	+
	Urease	*Rhodobacter sphaeroides*	22958664	22958703	-	-	+	+
	Urease	*Xylella fastidiosa*	22997051	23000613	+	+	+	+
	Urease	*Xylella fastidiosa*	22997051	23000614	-	+	+	+
	Urease	*Agrobacterium tumefaciens*	17933925	17936278	+	+	+	+
	Urease	*Bacillus halodurans*	15612563	15612821	+	+	+	+
	Urease	*Corynebacterium efficiens*	25026556	25027555	+	+	+	+
	Urease	*Proteus mirabilis*	150914	150919	+	+	+	+
	Urease	*Synechococcus sp.* WH 7805	3659626	7388348	+	+	+	+
	Urease	*Synechococcus sp.* WH 8102	33864539	33866976	-	-	+	-
	Urease	*Thermosynechococcus elongatus*	22294033	22294257	+	+	+	+
	Urease	*Thermosynechococcus elongatus*	22294885	22295090	-	+	-	-
	Urease (putative)	*Escherichia coli* O157:H7	16445223	15800669	+	-	-	+
	Urease (putative)	*Streptomyces avermitilis*	29826540	29833650	+	+	-	+
	Urease (putative)	*Streptomyces avermitilis*	29826540	29833651	+	+	+	+
Nitrification								
Ammonia	Ammonia monooxygenase	uncultured bacterium	3283949	3283950	+	+	+	+
	Ammonia monooxygenase	unidentified bacterium	7595785	7595786	+	+	+	+
	Ammonia monooxygenase	*Nitrosococcus oceani*	3282844	3282846	-	-	+	+
Hydroxylamine	Hydroxylamine oxidoreductase	*Nitrosomonas europaea*	30248031	30249984	-	+	-	+
Denitrification								
Nitrate	Nitrate reductase	*Haloarcula marismortui*	18447795	18447796	+	+	-	+
	Nitrate reductase (putative)	uncultured bacterium	26278739	26278740	+	-	-	+
	Nitrate reductase (putative)	uncultured bacterium	26278827	26278828	+	-	+	-
	Nitrate reductase (putative)	uncultured bacterium	26278841	26278842	+	+	+	+

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Nitrate (*cont'd)*	Nitrate reductase (putative)	uncultured bacterium	29652555	29652556	+	+	+	+
	Nitrate reductase (putative)	uncultured bacterium	29652453	29652454	+	+	-	+
	Nitrate reductase (putative)	uncultured bacterium	32307902	32307903	+	+	+	+
	Nitrate reductase (putative)	uncultured bacterium	32307964	32307965	-	-	+	-
	Nitrate reductase (putative)	*Rahnella sp.* Lgg5.8	17385455	17385456	+	+	-	+
	Nitrate transporter	*Aquifex aeolicus*	2982921	2982930	-	+	-	-
Nitrite	Nitrite reductase	*Bradyrhizobium japonicum*	27355365	27355371	+	+	-	+
	Nitrite reductase	*Haloferax denitrificans*	30089159	30089160	+	+	-	+
	Nitrite reductase	*Hyphomicrobium zavarzinii*	3758829	3758830	+	-	+	+
	Nitrite reductase	*Pseudomonas chlororaphis*	287906	287907	+	+	+	+
	Nitrite reductase	uncultured organism	24415271	24415272	-	+	-	+
	Nitrite reductase (putative)	*lab clone*	N/A	N/A	-	+	-	-
	Nitrite reductase (putative)	*lab clone*	N/A	N/A	+	+	+	+
	Nitrite reductase (putative)	*lab clone*	N/A	N/A	+	-	-	+
	Nitrite reductase	uncultured bacterium	28542574	28542575	+	+	+	-
	Nitrite reductase	uncultured bacterium	28542614	28542615	+	-	-	+
	Nitrite reductase	uncultured bacterium	37999157	37999158	+	+	-	+
Nitric oxide	Nitric oxide reductase	uncultured bacterium	29466065	29466066	+	+	+	+
Nitrous oxide	Nitrous oxide reductase	*Paracoccus pantotrophus*	3057082	3057083	-	-	+	-
	Nitrous oxide reductase	uncultured soil bacterium	32478409	32478410	-	-	+	-
	Nitrous oxide reductase (putative)	uncultured soil bacterium	29125885	29125886	+	-	-	-
Sulfur cycle								
Sulfite	Dissimilatory (bi-)sulfite reductase	uncultured sulfate-reducing bacterium	14389150	14389151	-	+	-	+
	Dissimilatory (bi-)sulfite reductase	uncultured sulfate-reducing bacterium	14389188	14389189	-	-	-	+
	Dissimilatory sulfite reductase	Solar Lake mat clone50	6014506	6014526	-	+	+	+

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Sulfite *(cont'd)*	Dissimilatory sulfite reductase	uncultured bacterium	20142103	20142104	+	+	-	+
	Dissimilatory sulfite reductase	uncultured bacterium	22203361	22203362	+	-	-	+
	Dissimilatory sulfite reductase	uncultured phenanthrene mineralizing bacterium	13898426	13898427	-	-	+	-
	Sulfite reductase	*Alvinella pompejana symbiont*	5006482	5006483	-	-	-	+
	Sulfite reductase	*Archaeoglobus fulgidus*	142188	142189	-	-	-	+
	Dissimilatory sulfite reductase	*Bilophila wadsworthia*	13992709	13591679	+	+	-	+
	Dissimilatory sulfite reductase	*Desulfofaba gelida*	15077476	15077478	+	+	+	+
	Dissimilatory sulfite reductase	*Desulfotomaculum kuznetsovii*	14276804	14276806	-	+	-	+
	Dissimilatory sulfite reductase	*Desulfotomaculum kuznetsovii*	13560083	13560085	+	+	+	+
	Dissimilatory sulfite reductase	*Desulfotomaculum kuznetsovii*	13560083	13560085	+	+	+	+
	Dissimilatory sulfite reductase	*Pelotomaculum sp.* MGP	40313559	40313561	+	+	-	+
	Dissimilatory sulfite reductase	uncultured sulfate-reducing bacterium	13249546	13249547	-	+	+	-
	Dissimilatory sulphite reductase (putative)	lab clone	N/A	N/A	-	-	-	+
	Dissimilatory sulphite reductase (putative)	lab clone	N/A	N/A	+	+	+	+
	Dissimilatory sulphite reductase (putative)	lab clone	N/A	N/A	-	-	-	-
	Dissimilatory sulphite reductase (putative)	lab clone	N/A	N/A	+	+	-	+
	Dissimilatory sulphite reductase (putative)	lab clone	N/A	N/A	+	+	+	+
Organic contaminant degradation								
2,4-D	LysR-type transcriptional regulator	*Wautersia eutropha*	1764154	567073	+	+	+	-
3-chlorobenzoate	Oxidoreductase (putative)	*Streptomyces coelicolor*	32141095	21218826	+	+	+	+
Acetylene	Acetylene hydratase	*Pelobacter acetylenicus*	33325844	33325847	-	-	+	+
	Epoxide hydrolase	*Mesorhizobium loti*	13470324	13475577	+	+	+	-

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Acetylene *(cont'd)*	Epoxide hydrolase (putative)	*Pirellula sp.* 1	32470666	32473431	+	+	+	+
Acrylonitrile	Nitrilase	uncultured organism	40890072	40890073	+	-	+	+
	Nitrilase	uncultured organism	40890078	40890079	+	+	+	+
	Nitrilase	uncultured organism	40890128	40890129	+	-	+	+
	Nitrilase	uncultured organism	40890140	40890141	+	+	+	+
	Nitrilase	uncultured organism	40890170	40890171	+	-	+	+
	Nitrilase	uncultured organism	40890312	40890313	-	+	+	+
	Nitrilase	uncultured organism	40890322	40890323	+	+	-	+
	Nitrile hydratase	uncultured bacterium	27657374	27657375	-	+	-	+
Amino-cyclopropane	1-aminocyclopropane-1-carboxylate deaminase	*Caulobacter crescentus*	16124256	16126275	-	-	-	+
	1-aminocyclopropane-1-carboxylate deaminase	*Pseudomonas sp.* 6G5	150964	231457	+	-	+	+
	1-aminocyclopropane-1-carboxylate deaminase	*Pyrococcus furiosus*	18976372	18976382	+	+	+	+
Aniline	Aniline degradation	*Acinetobacter sp.* YAA	2627146	2627150	+	+	+	+
	Aniline dioxygenase	*Acinetobacter sp.* YAA	2627146	2627148	+	+	+	-
	Dioxygenase	*Pseudomonas putida*	14715441	1841362	+	-	+	+
Atrazine	Atrazine chlorohydrolase	*Methanocaldococcus jannaschii*	15668172	15668880	+	+	+	+
	Atrazine chlorohydrolase (putative)	*Archaeoglobus fulgidus*	11497621	11499364	-	-	+	-
	Chlorohydrolase (putative)	*Streptomyces coelicolor*	32141095	21221512	+	+	+	+
	Chlorohydrolase (putative)	*Yersinia pestis*	22123922	22126223	+	+	+	+
	n-ethylammeline chlorohydrolase	*Pyrococcus furiosus*	18976372	18977910	-	-	+	-
Benzene	Cis-chlorobenzene dihydrodiol dehydrogenase	*Ralstonia sp.* JS705	27802968	27802969	+	-	+	-
Benzoate	Benzoate transport	*Acinetobacter sp.* ADP1	17865389	2352826	-	+	-	-

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Benzoate *(cont'd)*	Benzoate transport	*Pseudomonas putida*	7542431	7542440	+	+	-	+
	[2Fe-2S] ferredoxin	*Leptospirillum ferrooxidans*	31747628	31747634	+	+	+	+
	4-hydroxybenzoyl-CoA reductase	*Rhodopseudomonas palustris*	1730294	1730297	-	+	-	+
	Acetoacetyl CoA reductase	*Bradyrhizobium japonicum*	27375111	27375336	-	-	+	-
	Acetyl-CoA acetyltransferase	*Sulfolobus solfataricus*	15896971	15898850	-	+	+	+
	Acetyl-CoA acetyltransferase	*Sulfolobus solfataricus*	15896971	15899350	+	+	+	+
	Acetyl-CoA acetyltransferase	*Wautersia eutropha*	141953	135754	-	-	+	+
	Benzoate-CoA ligase	*Thauera aromatica*	23450980	23450983	+	+	+	-
	Benzoyl CoA reductase	*Azoarcus evansii*	18369654	18369658	+	-	+	+
	Benzoyl-CoA reductase	*Leuconostoc mesenteroides*	28876819	23054462	-	+	+	-
	Ferredoxin	*Campylobacter jejuni*	4105263	4105265	+	+	+	+
	Ferredoxin	*Desulfotomaculum thermocisternum*	4028016	4028019	+	+	-	+
	Ferredoxin	*Photorhabdus luminescens*	37524032	37527158	-	+	-	-
	Ferredoxin	uncultured bacterium	40063344	40063438	+	+	+	+
	Glutaryl-CoA dehydrogenase	*Bordetella bronchiseptica*	33598993	33599653	+	-	-	+
	Thiolase	*Bacillus halodurans*	15612563	15614560	+	-	-	+
	Thiolase	*Bacillus halodurans*	15612563	15614592	-	-	+	+
Benzonitrile	Amidase (putative)	*Rhodopseudomonas palustris*	39933080	39934770	-	-	+	-
	Nitrile hydratase	*Pseudomonas putida*	3172137	1877505	-	-	+	-
	Nitrile hydratase	*Rhodococcus erythropolis*	37221877	37221879	+	-	-	-
	Nitrile hydratase	*Rhodococcus sp.* N-771	4126493	4126495	+	+	+	+
	Nitrile hydratase activator	*Xanthomonas campestris*	21229478	21229735	-	+	+	+
Biphenyl	2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate hydrolase	*Rhodococcus sp.* M5	1177720	1177721	-	-	-	+
	Biphenyl dihydrodiol dehydrogenase	*Bacillus sp.* JF8	32562909	32562914	+	-	+	+

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Biphenyl *(cont'd)*	Biphenyl dioxygenase	*Wautersia eutropha*	1890341	1890342	+	+	+	+
	Dihydrodiol dehydrogenase	*Ralstonia sp.* SBUG 290	28193600	28193601	-	-	+	-
	Dioxygenase.	*Pseudomonas pseudoalcaligenes*	151090	151091	+	+	+	+
	Hydrolase (putative)	*Bacillus sp.* JF8	32562909	32562911	-	-	-	+
Camphor	Camphor resistance	*Rhodopseudomonas palustris*	39933080	39934764	+	+	-	+
	Camphor resistance (putative)	*Yersinia pestis*	15979723	15979772	+	+	+	+
Carbazole	Meta cleavage enzyme	*Sphingomonas sp.* KA1	28201219	28201227	-	-	+	+
Catechol	Catechol 2,3 dioxygenase	uncultured bacterium	28556734	28556735	+	+	+	+
Catechol (meta-derivative)	2-hydroxy-6-oxohepta-2,4-dienoate hydrolase	*Helicobacter pylori*	15644634	15645359	-	+	-	+
	2-hydroxy-6-oxohepta-2,4-dienoate hydrolase	*Nostoc sp.* PCC 7120	17227497	17227812	+	+	-	+
	2-hydroxy-6-oxohepta-2,4-dienoate hydrolase	*Wolinella succinogenes*	34556458	34556864	+	+	+	+
	4-oxalocrotonate decarboxylase	*Comamonas testosteroni*	6624267	6624275	-	-	+	+
	Carboxymethylene-butenolidase	*Caulobacter crescentus*	16124256	16127988	-	-	+	-
	Carboxymethylene-butenolidase (putative)	*Aquifex aeolicus*	2984235	3913489	+	+	+	+
Catechol (ortho-derivative)	Carboxymethylene-butenolidase (putative)	*Synechococcus sp.* WH 8102	33864539	33864628	-	+	-	-
	Dienelactone hydrolase	*Pseudomonas resinovorans*	27228492	27228503	-	+	-	+
	Dienelactone hydrolase	*Rhodococcus opacus*	2935030	2935034	+	+	+	+
	Dienelactone hydrolase (predicted)	*Pseudomonas syringae*	28876508	23471854	+	+	-	+
	Dienelactone hydrolase (predicted)	*Rhodobacter sphaeroides*	22959343	22966941	+	+	-	-
	Dienelactone hydrolase (putative)	*Mycobacterium leprae*	15826865	15827757	+	+	+	+

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Catechol (ortho) *(cont'd)*	Maleylacetate reductase (putative)	*Sulfolobus tokodaii*	24473558	15921941	+	+	+	+
	Muconate cycloisomerase	*Bacillus subtilis*	2293135	2293150	-	-	-	+
	Muconate cycloisomerase	*Oceanobacillus iheyensis*	23097455	23100298	+	+	+	+
	Muconate cyclo-isomerase (putative)	*Sinorhizobium meliloti*	16262453	16263250	+	+	+	-
	Muconate cyclo-isomerase (putative)	*Yersinia pestis*	22123922	22125883	-	-	-	+
Chloroacrylic acid	Alcohol dehydrogenase (putative)	*Pseudomonas pavonaceae*	10637968	10637972	+	-	+	-
Chlorophenol	Transcription regulator (putative)	*Desulfitobacterium dehalogenans*	7710204	7710209	+	+	+	-
	Unknown	*Desulfitobacterium dehalogenans*	7710204	7710207	-	+	+	+
Cyanuric acid	Allophanate hydrolase	*Azotobacter vinelandii*	29358938	23102160	+	+	+	+
	Allophanate hydrolase	*Haemophilus somnus*	32029906	32029922	+	+	+	-
	Allophanate hydrolase	*Pseudomonas syringae*	28876500	23472887	+	+	+	+
	Allophanate hydrolase	*Rhodobacter sphaeroides*	22957025	22957043	+	+	+	+
	Allophanate hydrolase	*Thermoanaerobacter tengcongensis*	20806542	20808036	+	+	+	+
Cyclohexanol	6-hydroxyhexanoate dehydrogenase	*Brevibacterium sp.* HCU	14719378	14719379	+	-	+	-
	Alcohol dehydrogenase	*Acinetobacter sp.* SE19	9965281	9965293	-	+	-	+
	Aldehyde dehydrogenase	*Acinetobacter sp.* SE19	9965281	9965287	+	+	+	+
	Cyclohexanone monooxygenase	*Bradyrhizobium japonicum*	27375111	27382095	-	+	-	+
Cyclopentanol	5-valerolactone hydrolase	*Comamonas sp.* NCIMB 9872	24460032	24460039	-	-	+	-
	Cyclopentanol dehydrogenase	*Comamonas sp.* NCIMB 9872	24460032	24460042	+	-	+	+
Cymene	Outer membrane protein	*Pseudomonas putida*	2228230	2228236	-	+	-	-
Dibenzothiophene	Cytochrome c (putative)	*Thiobacillus sp.* KCT001	14149087	14149089	-	+	+	+

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Dibenzothiophene *(cont'd)*	Dibenzothiophene desulfurization reductase	*Rhodococcus erythropolis*	11323311	11323312	+	+	+	+
	NAD(P)H-FMN oxidoreductase	*Bacillus subtilis*	40316487	40316488	+	-	+	+
	Thermophilic dibenzothiophene desulfurization	*Paenibacillus sp.* A11-2	45126774	7637394	-	+	-	+
	Thiosulfate-oxidizing enzyme	bacterium HY-86/2	26985955	26985956	-	-	-	+
	Thiosulfate-oxidizing enzyme	*Marinobacter sp.* HY-106	26986065	26986066	+	-	+	+
Dichloroethane	Haloalkane dehalogenase	*Bradyrhizobium japonicum*	27375111	27376198	+	+	+	+
	Haloalkane dehalogenase	*Nostoc sp.* PCC 7120	17227497	17227689	+	+	+	+
	Haloalkane dehalogenase	*Xylella fastidiosa*	15836605	15838559	-	-	+	-
Dimethylsulfoxide	Anaerobic dimethylsulfoxide reductase (putative)	*Salmonella typhimurium*	16763390	16767556	-	-	+	-
Dodecyl sulfate	Arylsulfotransferase	*Pseudomonas putida*	6019465	6019467	+	-	+	+
Gallate	Pyrogallol hydroxytransferase	*Pelobacter acidigallici*	5531320	41019498	+	+	+	+
g-hexachlorocyclo-hexane	2,5-dichloro-2,5-cyclohexa-diene-1,4-diol dehydrogenase	*Methanosarcina acetivorans*	20088899	20089308	+	+	+	+
	2,5-dichloro-2,5-cyclo-hexa-diene-1,4-diol dehydrogenase	uncultured bacterium	40062851	40062882	-	-	-	+
	2,5-dichloro-2,5-cyclo-hexa-diene-1,4-diol dehydrogenase	*Yersinia pestis*	22123922	22126596	+	-	+	-
	Haloalkane dehalogenase	*Mycobacterium tuberculosis*	15607142	15609716	-	-	-	+
Haloacid	2-haloacid dehalogenase H-109	*Pseudomonas putida*	402522	440136	+	+	-	+
	2-haloalkanoic acid dehalogenase (putative)	*Streptomyces coelicolor*	32141095	21218946	+	+	+	-
Homogentisate	Homogentisate 1,2-dioxygenase	*Pseudomonas putida*	26986745	26991305	-	-	-	+

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Homogentisate *(cont'd)*	Homogentisate 1,2-dioxygenase	*Sinorhizobium meliloti*	15963753	15966692	-	+	+	+
	Homogentisate 1,2-dioxygenase	*Xanthomonas campestris*	21229478	21229915	-	-	+	-
Limonene	Cyclase/dehydrogenase (putative)	*Rhodococcus erythropolis*	14331011	14331012	+	-	+	-
Methyl tert-butyl ether	Alkane 1-monooxygenase	*Prauserella rugosa*	13872674	13872675	+	+	+	+
	Alkane-1-monooxygenase	*Rhodococcus erythropolis*	13750756	13750758	+	+	-	+
Naphthalene	2-hydroxychromene-2-carboxylate isomerase	*Agrobacterium tumefaciens*	17938588	17939008	+	+	-	+
	2-hydroxychromene-2-carboxylate isomerase	*Nostoc punctiforme*	30581875	23129090	+	+	+	+
	2-hydroxychromene-2-carboxylate isomerase (putative)	*Ralstonia solanacearum*	17544719	17544992	+	-	-	-
	2-hydroxychromene-2-carboxylate isomerase family protein	*Bordetella bronchiseptica*	33598993	33603603	+	+	+	+
	Salicylaldehyde dehydrogenase	*Pseudomonas sp.* ND6	42632299	38638560	-	+	-	+
Nicotine	6-hydroxy L-nicotine oxidase	*Arthrobacter oxydans*	18958170	18958172	+	+	+	+
Nitrilotriacetate	Oxidoreductase	EDTA-degrading bacterium	9957194	9957198	-	+	+	+
Nitrobenzoate	P-nitrobenzoate reductase	*Pseudomonas sp.* YH102	6014662	6014664	-	-	-	+
	P-nitrobenzoate reductase	*Ralstonia pickettii*	6014658	6014661	+	+	+	+
Nitrotoluene	Benzenetriol oxygenase (putative)	*Burkholderia cepacia*	17942383	17942394	+	+	+	+
Parathion	Methyl parathion hydrolase	*Xanthomonas axonopodis*	21240774	21241497	+	+	-	+
Pentachlorophenol	LysR type transcriptional regulator (putative)	*Sphingobium chlorophenolicum*	22417091	22417104	-	-	-	+
Phenol	Phenol 2-mono-oxygenase-like protein	*Neurospora crassa*	18375977	18375992	-	+	-	+

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Phenylpropionate	3-(2,3-dihydroxyphenyl-propionate)1, 2-dioxygenase	*Escherichia coli*	1702879	1702882	-	+	-	+
	Phenylpropionate dioxygenase	*Azotobacter vinelandii*	29359385	23107057	+	+	+	+
	Phenylpropionate dioxygenase	*Azotobacter vinelandii*	29359385	23107605	+	-	+	+
	Phenylpropionate dioxygenase	*Azotobacter vinelandii*	29359385	23109674	+	+	+	+
	Phenylpropionate dioxygenase	*Leuconostoc mesenteroides*	28876819	23041321	-	-	+	+
	Phenylpropionate dioxygenase	*Leuconostoc mesenteroides*	28876819	23042515	+	-	+	+
	Phenylpropionate dioxygenase	*Leuconostoc mesenteroides*	28876819	23043434	+	+	+	+
	Phenylpropionate dioxygenase	*Nostoc punctiforme*	30581835	23126645	-	+	-	+
	Phenylpropionate dioxygenase	*Rhodobacter sphaeroides*	22959343	22975203	-	-	+	+
Phthalate	3,4-dihydroxy-3,4-dihydrophthalate dehydrogenase	*Terrabacter sp.* DBF63	27531091	27531096	-	+	-	-
	3,4-dihydroxyphthalate 2-decarboxylase	*Arthrobacter keyseri*	13242040	13242058	+	+	+	+
	4-methyl-o-phthalate permease (putative)	*Sulfolobus tokodaii*	24473558	15922956	+	+	-	+
	Phthalate 4,5-dioxygenase-like protein	*Neurospora crassa*	28950002	28950015	-	-	-	+
	Phthalate dioxygenase	*Arthrobacter keyseri*	13242040	13242054	-	-	+	-
	Phthalate dioxygenase	*Mycobacterium vanbaalenii*	49072885	37518573	+	+	+	+
	Phthalate ester hydrolase (putative)	*Arthrobacter keyseri*	13242040	13242052	+	+	+	+
Protocatechuate	Protocatechuate 3,4 dioxygenase (putative)	marine alpha proteobacterium SE45	38490063	38490070	+	+	+	+
	Protocatechuate 3,4 dioxygenase (putative)	*Ralstonia solanacearum*	17544719	17546160	+	+	+	+
	Protocatechuate 3,4-dioxygenase	*Leuconostoc mesenteroides*	28876819	23062663	+	+	+	+
	Protocatechuate 3,4-dioxygenase	*Leuconostoc mesenteroides*	28876819	23063177	+	+	+	+

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Protocatechuate *(cont'd)*	Protocatechuate 3,4-dioxygenase	*Rhodobacter sphaeroides*	22959343	22975204	+	+	+	+
	Protocatechuate 3,4-dioxygenase	*Caulobacter crescentus*	16124256	16126648	+	+	+	+
	Protocatechuate 3,4-dioxygenase	*Xanthomonas campestris*	21229478	21230278	+	+	-	+
	Protocatechuate 3,4-dioxygenase	*Corynebacterium glutamicum*	23308765	19553596	-	-	-	+
	Protocatechuate 3,4-dioxygenase	*Xanthomonas axonopodis*	21240774	21241648	-	-	+	+
	Protocatechuate 3,4-dioxygenase	*Bradyrhizobium japonicum*	27375111	27376038	-	-	-	+
Pyrene	Dioxygenase	*Mycobacterium gilvum*	26080262	26080263	+	+	+	+
	PAH ring-hydroxylating dioxygenase	*Mycobacterium sp.* 6PY1	27657407	27657409	-	-	+	-
	PAH ring-hydroxylating dioxygenase	*Mycobacterium sp.* 6PY1	27657412	27657413	-	-	-	+
Salicylate	Putative salicylate hydroxylase	*Aspergillus fumigatus*	19309393	19309419	+	-	-	+
	Salicylate hydroxylase	*Pseudomonas fluorescens*	38004624	38004625	-	-	-	+
Thiocyanate	Acetyl-CoA decarbonylase/synthase	*Methanopyrus kandleri*	19887164	38503097	+	+	+	+
	Carbon monoxide dehydrogenase	*Geobacter sulfurreducens*	39995111	39997196	-	+	-	+
	Carbon monoxide dehydrogenase	*Methanocaldococcus jannaschii*	1591436	38257392	+	+	-	+
	Carbon monoxide dehydrogenase	*Rhodopseudomonas palustris*	39933080	39936863	-	-	-	+
	Carbon monoxide dehydrogenase	*Sulfolobus solfataricus*	15896971	15898062	-	+	+	+
	Carbon monoxide dehydrogenase	*Sulfolobus solfataricus*	15896971	15899362	+	+	-	+

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Thiocyanate *(cont'd)*	Carbon monoxide dehydrogenase	*Thermoproteus tenax*	41033718	41033719	+	+	+	+
	Cyanate hydratase	*Aquifex aeolicus*	2982921	20137698	-	+	-	+
	Cyanate hydratase	*Pseudomonas aeruginosa*	9948050	20137979	+	-	+	+
Toluene	Benzyl alcohol dehydrogenase (putative)	*Corynebacterium efficiens*	25026556	25027545	+	+	+	+
	Anaerobic toluene metabolism protein	*Thauera aromatica*	29837127	3184127	+	-	-	+
	Benzylsuccinate synthase	*Thauera aromatica*	29837127	3184130	-	+	+	+
	Benzylsuccinate synthase	*Thauera aromatica*	29837127	3184132	-	+	-	-
	Pyruvate formate-lyase	*Clostridium tetani*	28209834	28210648	+	-	+	-
	Sensor kinase	*Azoarcus sp.*	17223649	17223651	+	+	+	+
Vanillin	Vanillate o-demethylase (probable)	*Ralstonia solanacearum*	17548221	17548444	-	-	+	-
	Vanillate O-demethylase oxidoreductase	*Bradyrhizobium japonicum*	27375111	27378510	+	+	-	+
	Vanillate O-demethylase oxygenase (putative)	*Corynebacterium efficiens*	25026556	25027190	+	+	+	-
	Vanillate-O-demethylase	*Pseudomonas sp.*	1946283	1946284	+	+	-	+
Xylene	3-ketoacyl-(acyl-carrier-protein) reductase	*Pseudomonas putida*	7804934	7804936	+	+	+	+
Metal reduction/resistance								
Arsenic	Arsenate reductase	*Bradyrhizobium japonicum*	27353135	27353339	+	-	-	-
	Arsenate reductase	*Deinococcus radiodurans*	15807672	15807792	-	+	-	+
	Arsenate reductase	*Mycobacterium tuberculosis*	15607142	15609822	+	-	+	+
	Arsenate reductase	plasmid R773	151856	151858	+	-	-	+
	Arsenate reductase	*Pseudomonas putida*	26986745	26989435	+	+	+	+
	Arsenate reductase (putative)	*Pirellula sp.* 1	32470666	32476814	+	+	+	-
	Arsenic resistance (putative)	*Ralstonia solanacearum*	17548221	17549350	+	+	+	+

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Arsenic *(cont'd)*	Arsenic resistance	*Clostridium acetobutylicum*	15004705	15004806	+	+	+	+
	Arsenite efflux pump	*Desulfitobacterium hafniense*	30471365	23112233	-	+	-	-
	Arsenite efflux pump	*Rhodobacter sphaeroides*	22959343	22960769	+	+	+	-
	Arsenite efflux pump	*Magnetospirillum magnetotacticum*	23011659	23011662	-	-	+	-
	Arsenite transporter (putative)	*Synechococcus sp.* WH 8102	33864539	33865573	+	-	-	+
	Thiopurine s-methyltransferase	*Pseudomonas putida*	26986745	26988600	+	+	+	+
Bismuth	Metal binding polypeptide	*Helicobacter pylori*	836666	836667	-	-	-	+
Cadmium	Cadmium resistance ATPase	*Bacillus sp.* MSA 235	28627879	28627880	-	-	-	+
	Cadmium resistance protein (predicted)	*Leuconostoc mesenteroides*	28876868	23023727	-	-	+	+
	Cadmium-exporting ATPase (putative)	*Helicobacter felis*	6465952	6465953	+	-	+	-
	Cation efflux system protein	*Xanthomonas axonopodis*	21240774	21244878	+	+	-	+
	Cation efflux system protein	*Xanthomonas axonopodis*	21240774	21244879	-	-	+	+
	Metal resistance protein (hypothetical)	*Corynebacterium glutamicum*	23308765	19552133	-	+	+	+
	Metal resistance protein (hypothetical)	*Wolinella succinogenes*	34556458	34557205	-	-	+	-
	Cadmium resistance	*Pseudomonas syringae*	28867243	28872392	+	+	+	-
Chromium	Chromate ion transporter (putative)	*Chromobacterium violaceum*	34495455	34496726	-	+	-	+
	Chromate resistance	*Methanocaldococcus jannaschii*	15668172	15668899	+	+	+	-
	Chromate resistance (putative)	*Synechococcus sp.*	687686	687689	+	+	+	+
	Chromate transport	*Synechocystis sp.* PCC 6803	16329170	16332117	+	+	+	+
	Chromate transport	*Pseudomonas aeruginosa*	32037442	32039492	-	-	+	-
	Chromate transport	*Leuconostoc mesenteroides*	28876819	23055123	+	+	+	+
	Chromate transport	*Magnetospirillum magnetotacticum*	23012808	23012809	+	-	-	-
	Chromate transport	*Nostoc punctiforme*	30581753	23123941	+	+	+	+

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Cobalt, zinc, cadmium	Cation-efflux transporter	*Corynebacterium diphtheriae*	38232642	38233695	-	+	-	-
	Cation-efflux transporter (putative)	*Mycobacterium leprae*	15826865	15827060	+	+	+	-
	Metal resistance protein (hypothetical)	*Geobacillus stearothermophilus*	1944408	1944409	-	+	-	+
	Metal resistance protein (hypothetical)	*Wautersia metallidurans*	1731912	1731913	+	+	-	+
Copper	ATPase	*Enterococcus hirae*	290641	290643	+	+	+	+
	Cation efflux transporter (putative)	*Legionella pneumophila*	19880988	19881004	-	-	-	+
	Cation transporting ATPase	*Sulfolobus solfataricus*	15896971	15899375	+	-	-	+
	Copper resistance (uncharacterized)	*Lactobacillus gasseri*	28877238	23003052	+	+	+	-
	Copper resistance (uncharacterized)	*Rhodobacter sphaeroides*	22959343	22982455	+	-	-	-
	Copper resistance (uncharacterized)	*Azotobacter vinelandii*	29359385	23109144	-	-	+	-
	Copper resistance (uncharacterized)	*Leuconostoc mesenteroides*	28876819	23026822	+	+	+	+
	Copper resistance	*Aeromonas veronii*	6941899	6941901	+	+	+	-
	Copper resistance	*Helicobacter felis*	2660538	2660542	+	+	+	+
	Copper resistance	*Pseudomonas fluorescens*	14331036	14331038	-	+	-	+
	Copper resistance	*Pseudomonas putida*	32815822	32815823	-	-	+	-
	Copper resistance	*Pseudomonas syringae*	151187	151188	+	-	-	-
	Copper resistance	*Serratia marcescens*	38347830	38347977	+	-	-	+
	Copper resistance	*Wautersia metallidurans*	10047060	10047068	-	-	-	+
	Copper resistance (putative)	*Ralstonia solanacearum*	17548221	17548880	-	-	-	+
	Copper resistance transcriptional activator	*Pseudomonas putida*	26986745	26992059	+	+	+	+

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Copper *(cont'd)*	Copper resistance transcriptional activator	*Yersinia pestis*	16120353	16122243	+	+	-	+
	Copper-transporting ATPase (putative)	uncultured bacterium	37222109	37222119	+	+	-	+
	Superoxide dismutase	*Haloferax volcanii*	149032	149033	+	+	+	+
cytochrome	Cytochrome c biogenesis	*Leuconostoc mesenteroides*	28876819	23054496	-	-	+	-
	Cytochrome c biogenesis	*Leuconostoc mesenteroides*	28876819	23055461	-	-	-	+
	Cytochrome c precursor	metagenome clone	N/A	N/A	-	-	+	-
	Cytochrome	*Anaeromyxobacter dehalogenans*	N/A	N/A	+	+	-	+
	Cytochrome	*Anaeromyxobacter dehalogenans*	N/A	N/A	-	-	-	+
	Cytochrome	*Anaeromyxobacter dehalogenans*	N/A	N/A	+	+	-	+
	Cytochrome	*Anaeromyxobacter dehalogenans*	N/A	N/A	-	-	+	-
	Cytochrome	*Desulfitobacterium hafniense*	N/A	N/A	+	+	+	+
	Cytochrome	*Desulfitobacterium hafniense*	N/A	N/A	-	-	-	+
	Cytochrome	*Desulfitobacterium hafniense*	N/A	N/A	+	+	+	+
	Cytochrome	*Desulfitobacterium hafniense*	N/A	N/A	+	-	-	+
	Cytochrome	*Desulfovibrio desulfuricans*	N/A	N/A	+	+	+	+
	Cytochrome	*Desulfovibrio desulfuricans*	N/A	N/A	+	+	+	+
	Cytochrome	*Geobacter metallireducens*	N/A	N/A	-	-	-	+
	Cytochrome	*Geobacter metallireducens*	N/A	N/A	+	+	-	-
	Cytochrome	*Geobacter metallireducens*	N/A	N/A	+	+	+	+
	Cytochrome	*Geobacter metallireducens*	N/A	N/A	+	+	+	+
	Cytochrome	*Geobacter metallireducens*	N/A	N/A	-	-	-	+
	Cytochrome	*Geobacter metallireducens*	N/A	N/A	+	+	+	+
	Cytochrome	*Leuconostoc mesenteroides*	28876819	23054433	+	+	+	+
	Cytochrome	*Desulfovibrio desulfuricans*	28877446	23475586	-	-	+	-
	Cytochrome	*Geobacter sulfurreducens*	39995111	39995807	+	+	-	-
	Cytochrome	*Geobacter sulfurreducens*	39995111	39997301	-	-	+	-
	Cytochrome	*Geobacter sulfurreducens*	39995111	39997527	+	+	+	+

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Cytochrome *(cont'd)*	Cytochrome	*Geobacter sulfurreducens*	39995111	39997596	+	-	+	-
	Cytochrome	*Geobacter sulfurreducens*	39995111	39997739	+	+	+	+
	Cytochrome	*Geobacter sulfurreducens*	39995111	39997901	+	+	+	+
	Cytochrome	*Geobacter sulfurreducens*	39995111	39997978	+	+	+	+
	Cytochrome	*Geobacter sulfurreducens*	39995111	39998004	-	-	+	-
	Cytochrome	*Geobacter sulfurreducens*	39995111	39998021	+	+	+	+
	Cytochrome	*Geobacter sulfurreducens*	39995111	39998304	+	+	+	+
	Cytochrome	*Geobacter sulfurreducens*	39995111	39998363	+	+	+	+
	Cytochrome	*Geobacter sulfurreducens*	39995111	39997989	-	+	-	+
	Cytochrome	metagenome clone	N/A	N/A	-	+	-	+
	Cytochrome	*Desulfovibrio desulfuricans*	18073934	18073936	+	-	-	-
	Cytochrome	*Geobacter sulfurreducens*	39995111	39995331	-	-	+	-
	Cytochrome	*Shewanella oneidensis*	24371600	24376080	+	+	+	+
	Cytochrome	*Shewanella oneidensis*	24371600	24372308	+	+	+	+
Lead	Lead resistance	*Wautersia metallidurans*	12718498	12718504	+	+	+	-
	Lead resistance	*Wautersia metallidurans*	12718498	12718500	+	+	+	+
	Lead resistance (putative)	*Pirellula sp.* 1	32470666	32477238	+	+	+	+
Metals	Multidrug resistance protein	metagenome clone	N/A	N/A	+	+	+	+
	Quinone oxidoreductase	metagenome clone	N/A	N/A	+	+	-	+
Mercury	Mercuric reductase	*Gloeobacter violaceus*	37519569	37520133	-	+	-	+
	Mercuric resistance	*Xanthomonas campestris*	6689524	6689528	-	+	-	-
	Mercuric resistance	metagenome clone	N/A	N/A	+	-	+	+
	Mercuric resistance	*Pseudomonas sp.* K-62	4115575	4115578	+	+	+	+
	Mercury transport	*Pseudomonas stutzeri*	2947082	2947088	-	+	-	+
	Mercury (II) reductase	*Halobacterium sp.* NRC-1	15789340	15789469	+	-	-	+
	Mercuric ion binding protein	*Pseudomonas sp.*	18076022	18076026	-	+	+	-
	Transcriptional regulator (putative)	*Neisseria meningitidis*	7380091	7380159	-	-	+	-

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Nickel	Nickel transporter	*Pseudomonas putida*	26986745	26990061	-	+	-	+
	Nickel resistance	*Synechocystis sp.* PCC 6803	16329170	16331903	+	+	+	+
	Nickel resistance	*Nostoc sp.* PCC 7120	17158637	17158765	+	+	+	+
	Nickel resistance	*Ruegeria sp.* PR1b	28558826	28558906	-	+	-	+
	Nickel transporter	*Helicobacter hepaticus*	32265499	32265913	+	+	+	+
	Nickel transporter	*Escherichia coli* K12	49175990	16131350	+	+	+	+
Nickel, cobalt, cadmium	Nickel, cobalt, cadmium resistance	*Helicobacter pylori*	15644634	15645585	+	+	+	+
	Nickel, cobalt, cadmium resistance	metagenome clone	N/A	N/A	-	+	-	+
Tellurium	Metal resistance protein (hypothetical)	*Rhodobacter sphaeroides*	22956532	22956535	+	+	+	+
	Metal resistance protein (hypothetical)	*Vibrio vulnificus*	27366463	27366689	-	-	+	-
	Tellurite resistance	*Escherichia coli*	26245917	26247714	+	-	-	+
	Tellurite resistance	*Xylella fastidiosa*	22997051	23000799	-	+	-	+
	Tellurite resistance	*Rhodobacter sphaeroides*	2209124	2314827	-	-	+	-
	Tellurite resistance	*Deinococcus radiodurans*	15805042	15807217	+	-	-	-
	Tellurite resistance	*Rhodobacter sphaeroides*	22959343	22981959	-	-	-	+
	Tellurite resistance	*Deinococcus radiodurans*	15805042	15807661	-	+	+	+
	Tellurite resistance (putative)	*Actinobacillus pleuropneumoniae*	32035517	32035774	-	+	+	-
	Tellurite resistance (putative)	*Azotobacter vinelandii*	29359345	23105653	+	+	-	+
	Tellurite resistance (putative)	*Desulfitobacterium hafniense*	30471338	23112224	+	+	+	+
	Tellurite resistance (putative)	*Lactobacillus gasseri*	28877231	23003390	-	-	-	+
	Tellurite resistance (putative)	*Leuconostoc mesenteroides*	28876819	23053132	-	+	-	+
	Tellurite resistance (putative)	*Leuconostoc mesenteroides*	28876819	23055848	+	+	+	+
	Tellurite resistance (putative)	*Pseudomonas resinovorans*	27228492	27228548	-	+	+	+
	Tellurite resistance (putative)	*Streptomyces avermitilis*	29826540	29832347	-	-	+	-

TABLE A-1. Continued

Target	Gene description	Biological origin	Genbank ID		Detection			
			Nucleotide	Protein	M	C_3	4S	4M
Perchlorate metabolism								
Perchlorate	Chlorate metabolism	*Ideonella dechloratans*	34494347	34494351	+	+	+	+
	Chlorate reductase	*Ideonella dechloratans*	34494347	34494349	+	+	-	+
	Chlorite dismutase	*Dechloromonas agitata*	22212276	22212277	+	+	-	-

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- 1st place Graduate Student Poster, Div. S-3, Soil Science Society of America (2006)
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Selected presentations and abstracts:

- Hollister EB, Boutton TW, Schadt CW, and Palumbo AV. 2007. Soil microbial diversity in a mesquite savanna. 11th Biennial Meeting of the Soil Ecology Society, Moab, UT.
- Hollister EB, Boutton TW, and Ansley RJ. 2006. Land use and land cover changes in temperate savannas: Impact of woody encroachment and prescribed burning on soil carbon pools and flux rates. Annual Meeting of the Soil Science Society of America, Indianapolis, IA.
- Hollister EB, Boutton, TW, and Ansley RJ. 2006. Influence of fire and woody plant invasion on the carbon dynamics of a Southern Great Plains grassland system. Annual Meeting of the Ecological Society of America, Memphis, TN.
- Hollister EB, Boutton TW, and Ansley RJ. 2005. Carbon dynamics of temperate savannas: Effects on prescribed fire on microbial activity and potential carbon mineralization. Annual Meeting of the Society for Range Management, Fort Worth, TX.